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The Role of the APOE Gene in Cognitive Recovery Following Traumatic Brain Injury: An Exploration of Direct Effects and Interactions with Age and Sex.

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Submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

University of Tasmania, June 2016

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Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator, and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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Date: 8/6/16

Publications from Thesis

Padgett, C. R., Skilbeck, C. E., & Summers, M. J. (2014). Missing data: The importance and impact of missing data from clinical research. *Brain Impairment, 15*, 1-9.

Padgett, C. R., Summers, M. J., Vickers, J. C., McCormack, G. H., & Skilbeck, C. E. (2016). Exploring the effect of the apolipoprotein gene on executive function, working memory, and processing speed during the early recovery period following traumatic brain injury. *Journal of Clinical and Experimental Neuropsychology*. *38*(5), 551-560. doi 10.1080/13803395.2015.1137557

Padgett, C. R., Summers, M. J. & Skilbeck, C. E. (2016). Is apolipoprotein ε4 associated with poorer cognitive outcome following traumatic brain injury? A meta-analysis. *Neuropsychology.* Advance Online Publication. doi 610.1037/neu0000270

Papers currently submitted

Padgett, C. R., Summers, M. J., Honan, C. A., McCormack, G. H., Vickers, J. C., & Skilbeck, C. E. (under review). Does Apolipoprotein ε4 interact with age or sex in cognitive function after traumatic brain injury? *Brain. [BRAIN-2016-00203]*.

Abstracts

Padgett, C., Skilbeck, C. E., Summers, M. J., & Vickers, J. C. (2012). The role of APOE genotype in recovery following TBI: Is the ε4 allele associated with poorer outcome? *The abstracts of the 2012 International Neuropsychological Society Mid-Year Meeting/11th Nordic Meeting in Neuropsychology June 27-30, 2012 Oslo, Norway. Journal of the International Neuropsychological Society, 18,* S2, 108-115. doi 10.1017/S1355617712001075

Padgett, C., Skilbeck, C. E., & Summers, M. J. (2011). Are female sex hormones protective following traumatic brain injury? *The abstracts of the 2011 International Neuropsychological Society Mid-Year Meeting/ASSBI 4th Pacific Rim Conference. Journal of the International Neuropsychological Society, 17,* 52-55. doi 10.1017/S1355617711001159.

Refereed Conference Presentations

Padgett, C., Skilbeck, C. E., & Summers, M. J. (2013). *Missing data – to ignore or impute?* Symposium presentation at the Annual Conference for the Australasian Society for the Study of Brain Impairment, Hobart, Australia.

Padgett, C., Skilbeck, C. E., Summers, M. J., & Vickers, J. C. (2012). *Role of APOE genotype in recovery following TBI.* Podium presentation at the Australian Neurotrauma Symposium. Annual Conference, Hobart, Australia.

Padgett, C., Skilbeck, C. E., Summers, M. J., & Vickers, J. C. (2012). *The role of APOE genotype in recovery following TBI: Is the ε4 allele associated with poorer outcome?* Poster presentation at the International Neuropsychological Society Mid-Year Conference, Oslo, Norway.

Padgett, C., Skilbeck, C. E., & Summers, M. J. (2011). *Are female sex hormones protective following traumatic brain injury?* Poster presentation at the International Neuropsychological Society Mid-Year Conference, Auckland, New Zealand.

Padgett, C., Skilbeck, C. E., & Summers, M. J. (2011). *The role of the APOE gene in neuropsychological recovery following traumatic brain injury.* Podium presentation at the Cradle Coast Postgraduate Research Conference – Research into the Future. Burnie, Australia.

Abstract

Traumatic brain injury (TBI) is frequently associated with cognitive impairment, which can be either transient or lifelong. Despite this, the ability to predict who will experience poorer cognitive outcomes remains challenging. The APOE gene, which has three alleles; APOE ε2, ε3, and ε4, has recently attracted attention as a biomarker that may be of prognostic value, as the resultant protein is believed to facilitate post-injury repair. The protein derived from the APOE ε4 allele is less structurally sound than that of the ε2 or ε3 alleles and as such, it has been proposed that APOE ε4 carriers may have impaired recovery of cognitive function after injury. Although there is growing literature exploring this hypothesis, the findings to date have been inconclusive. This ambiguity may be due in part to methodological limitations, such as the small sample sizes typically associated with genetic studies in clinical populations, and also may be due to moderating factors being overlooked. For example, it has been proposed that APOE ε4 may have an antagonistic pleiotropic effect whereby it confers beneficial effects during the reproductive phase of life, and becomes detrimental during the post-reproductive life phase. Furthermore, there is evidence that female sex hormones enhance the expression of APOE, but that this may not occur for female APOE ε4 carriers due to the structural nature of the protein. However, the possible effects of age and sex are yet to be investigated in relation to post TBI cognitive function. The aims of the current thesis are twofold; firstly, to provide a more integrative and in-depth investigation of the relationship between APOE ε4 and TBI by conducting a meta-analysis of the literature to date, and secondly, to investigate whether factors such as age and sex may interact with the expression of APOE ε4 in relation to post-TBI cognitive function. This thesis comprises four studies: Firstly, a meta-analysis was conducted to provide a more comprehensive interpretation of the extant literature. This revealed no significant differences between APOE ε4 carriers and non-carriers, either in terms of general cognitive function or within specific

cognitive domains known to be commonly impacted by TBI. Limitations of the literature to date were identified and attempts were made to redress these in the following two studies, which both assessed the processing speed, working memory and executive function of participants who had sustained a TBI. One study assessed these functions during the acute recovery period by exploring the contribution of all three APOE alleles separately (N = 142; APOE ε4 = 37, APOE ε3 = 92, APOE ε2 = 13), and also considering whether injury severity influenced the expression of APOE ε4. This revealed that possession of APOE genotype was unlikely to contribute to differences, regardless of injury severity. The following study employed a longitudinal design, and measured outcomes at 3, 6 and 12 months post injury (N = 119; APOE ε4 = 30, APOE ε3 = 77, APOE ε2 = 12). In this study, as well as considering the general effect of APOE ε4, age and/or sex differences were evaluated to determine whether there was an interaction between these factors and APOE ε4. There was tentative evidence that APOE ε4 was associated with impaired executive function, but this was inconsistent. There was no evidence of an interaction between age and APOE ε4, and little evidence of an interaction between sex and APOE ε4. In an adjunct methodological study, the effect of missing data was explored in the sample and a number of traditional and more recently developed techniques used to compensate for missingness were applied to determine the ability of each to provide the best estimate of the true sample parameters. This thesis has a number of strengths, including the separate categorising of all three alleles, and the consideration of potential interactions between age, sex and APOE ε4. The key finding of this thesis is that APOE ε4 is unlikely to have a pervasive effect on cognitive recovery after TBI, but that further investigation of the interaction between APOE status and age and sex is needed.

Acknowledgements

I am immensely indebted to my supervisors; Clive Skilbeck, Mathew Summers, and Mike Garry. Clive, thank you so much for your advice and for welcoming me into the Neurotrauma Register team. You gave me the confidence to go down the research path I wanted to follow, and prompted me to step outside of my comfort zone at times. Not only did you give me so much support and advice in developing my thesis, you and Sandy have also been incredibly welcoming and generous, and made my trips to Hobart a delight. Mathew, you have always provided invaluable advice and encouragement, and given me so much of your time. Thank you so much for taking over when Clive retired. Despite us being in different states for the latter part of the thesis, I always knew I could rely on you for a quick reply to emails, and for meetings whenever needed. There were times when I couldn't see the forest (plots!?) for the trees, but you were able to remind me of the direction I needed to go. Mike, your advice on all things statistical has been greatly appreciated, and gave me confidence when I was uncertain which path to go, and I have always appreciated the support you have given me in my teaching as well. I am also indebted to James Vickers, Graeme McCormack, and Tracy Dickson at the Menzies for so kindly processing my DNA samples. Without your generosity I could not have undertaken the research I wanted to do, and I hope that one day I can return the favour in some way. Lastly, Cynthia Honan, thank you so for your time and advice on my analysis. I hope that we will have the chance to do some interesting work together.

To my office-mate and friend Merete, we have shared some incredible highs and lows while doing our theses. The benefit of getting to know you far outweighed the disadvantages of mostly working on a campus where the psychology department consisted of me alone. And thank you especially for the annual flödeboller whenever you returned from Denmark!

My work colleagues at Launceston and Hobart also deserve mention; Peter, Matt, Douglas and Cynthia in Launceston, and Rachel and Kim in Hobart especially. Thank you for all your advice in teaching and for being such great colleagues.

Thanks to my family and friends for understanding when I disappeared for months on end, and for providing me with much needed R & R breaks when time permitted. Hopefully you will still all remember me when I re-emerge from my office, blinking at the sunlight, in the near future.

Thank you to UTAS and the Australian Government for the financial support they provided, and to all the staff at the Neurotrauma Register. Collectively I hope we can contribute to a better understanding of traumatic brain injury. And to all my participants who so freely gave their time, thank you. I am never ceased to be impressed that so many people are willing to engage in research for purely altruistic reasons.

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List of Abbreviations

AD……..Alzheimer's Disease

apoE……Apolipoprotein E (protein)

APOE……...……..Apolipoprotein E (gene)

APOE ε2…….……Apolipoprotein E epsilon 2 allele

APOE ε3…….……Apolipoprotein E epsilon 3 allele

APOE ε4…….……Apolipoprotein E epsilon 4 allele

ARMS-PCR……...…Amplification refractory mutations system polymerase chain reaction

BDNF…….Brain-derived neurotropic factor

BVMT……...…… Brief Visuospatial Memory Test

CFT…….……Complex Figure Task

cGxE…….…..Candidate gene by environment interaction

COMT……Catechol-O-methyl transferase gene

COWAT……Controlled Oral Word Association Task

CVLT…….Californian Verbal Learning Task

DS…….……..Digit Span

DS-B…….……..... Digit Span Backwards

DS-F……..... Digit Span Forwards

DS-FB……...…….... Digit Span Forwards Backwards Ratio

ESCI……...…….……....Exploratory Software for Confidence Intervals Programme

FIML……..Full information maximum likelihood

FSIQ…….…..Full scale intelligence quota

GCS……Glasgow Coma Sale

GOAT…….Galveston Orientation and Amnesia Test

GOS……Glasgow Outcome Scale

GOS-E……Glasgow Outcome Scale Extended

IP Speed…….…….Information processing speed

KIBRA……..…. Kidney and brain expressed protein

LNS……..…Letter Number Sequencing

MAIB……..Motor Accidents Insurance Board

MAR……...Missing at random

MCAR……....Missing completely at random

MI……...Multiple imputation

ML……..Maximum likelihood

MNAR……....Missing not at random

NART…….……National Adult Reading Test

PTA…….……..……..Post traumatic amnesia

RAVLT……..……..…Rey Auditory Verbal Learning Task

RHH……...….Royal Hobart Hospital

SPSS……..…….Statistical Package for the Social Sciences

TBI……..……Traumatic Brain Injury

TMTB…….……Trail Making Task B

TNTR……..…...…….Tasmanian Neuro-Trauma Register

WAIS……. Wechsler Adult Intelligence Scale

WCST…….…..…..Wisconsin Card Sorting Task

WMI……..….Working Memory Index

WMS……..…Wechsler Memory Scale Logical Memory Task

Chapter 1: Introduction and Thesis Overview

Traumatic brain injury (TBI) is a relatively common injury, affecting millions of children and adults worldwide (Hyder, Wunderlich, Puvanachandra, Gururaj, & Kobusingye, 2007). Severity can range from temporary impairment of consciousness, to death, and for those who survive the initial injury, the damage sustained can result in chronic disability. There is also growing evidence that TBI is associated with increased risk of developing a number of disorders, including depression, epilepsy, Alzheimer's disease (AD), chronic traumatic encephalopathy, and Parkinson's disease (Masel & DeWitt, 2010). Therefore, TBI has the potential to exert serious direct and indirect life-long effects on an individual's well-being. Further, with the prevalence of TBI being highest during childhood and early adulthood, any resultant impairment can last decades, having devastating consequences for the sufferer and their families (Myburgh et al., 2008; Verhaeghe, Defloor, & Grypdonck, 2005). Sustaining a TBI can result in impairment to cognitive, emotional and motor function, and as such there is the potential for a wide range of negative outcomes, including physical disability, reduced capacity to work, difficulty in making responsible financial decisions and performing normal activities of daily living, and inability to engage in and maintain relationships (Hoofien, Gilboa, Vakil, & Donovick, 2001; Konrad et al., 2011; Morton & Wehman, 1995; Shames, Treger, Ring, & Giaquinto, 2007). The financial and emotional toll on the sufferer and their family members can be enormous, with an ongoing and significant financial burden and demand placed on the public health system (Kolakowsky-Hayner, Miner, & Kreutzer, 2001; Leibson et al., 2012; Mar et al., 2011; Ponsford, Olver, Ponsford, & Nelms, 2003; Tuominen, Joelsson, & Tenovuo, 2012).

Definition of TBI

Menon, Schwab, Wright, Maas, and Common (2010) describe TBI as "an alternation in brain function, or other evidence of brain pathology, caused by an external force" (2010, p 1638). According to this definition, for an alteration in brain function to be diagnosed, there needs to be at least one of the following; loss of, or reduced, consciousness, retrograde or posterior grade amnesia for the time of injury, neurological impairment, or change in mental state at time of injury. Brain pathology can also be used to diagnose TBI, and may incorporate neuroimaging, visual assessment, and/or testing for bio-markers associated with TBI. The external forces that inflict the injury can include collision of the head with an object, damage caused by acceleration/deceleration forces, penetration of the brain by a foreign object, or percussive injury, as inflicted by explosive forces. TBI excludes those brain injuries arising from other cerebral accidents such as stroke, damage due to hypoxia, substance abuse, or congenital impairment.

Estimating injury severity is often challenging. While diagnosis typically occurs at the time of injury it is possible, especially in cases of mild TBI, for the injury to remain undiagnosed until a considerable time after the event due to the subtle and/or delayed nature of some symptoms (Bazarian et al., 2009; Menon et al., 2010). This is particularly the case for psychological effects such as mood disorders, memory impairment, and executive dysfunction (Dean & Sterr, 2013; Vanderploeg, Curtiss & Belanger, 2005). It can also be difficult to establish length of time that length of coma (LOC) and/or post traumatic amnesia (PTA) have occurred. For example, if medical interventions such as mechanical intubation and/or anaesthetic drugs are administered, or if there are delays in the individual presenting for treatment (for example blast injuries in the military setting, or in rural settings), self-report is necessary to estimate injury severity (Menon et al., 2010; Roozenbeek, Maas, & Menon, 2013). Furthermore, some diagnostic tools routinely used at time of injury, such as CT scans,

may not be sufficiently sensitive to detect mild TBI, despite later detection of damage when MRI is used (Yuh et al., 2013). Additionally, there is evidence of inconsistency between scales used to measure injury severity, such as the Glasgow Coma Scale (GCS) which measures depth of coma, and length of coma and PTA estimates (Sherer, Struchen, Yablon, Wang, & Nick, 2008). As such, there is considerable heterogeneity in the methodology used to estimate injury making comparisons both between individuals and between scales difficult.

TBI Epidemiology – An Australian Perspective

Establishing accurate incidence and prevalence rates of TBI is challenging. There are a range of diagnostic criteria used, and it is likely that many individuals who sustain a mild TBI are not diagnosed or hospitalised, whilst others with severe injuries may die before being admitted to hospital. It is also not uncommon for an individual to sustain multiple TBIs over the course of their life (Fortune & Wen, 1999; Roozenbeek et al., 2013). With these limitations in mind, Fortune and Wen (1999) estimated that in Australia approximately 149 per 100,000 individuals are hospitalised annually with a diagnosis of TBI, based on hospitalisation data obtained in the financial year of 1996-1997. Approximately 70% of these cases were male, with injuries mostly occurring in paediatric cohorts (ages 0-4), young adults (15-19), and in the very old (85+), with the young adult group having the highest incident rate (284/100,000). Given the high frequency of TBIs in the young adult population, Harrison, Berry and Jamieson (2012) reviewed the incidence rates for Australians aged 15 to 24 years old. They report that hospitalisation for TBI in this age cohort was 169.3 per 100,000 of the population. It was also noted that males were 3.2 times more likely than females to experience TBI.

Myburgh and colleagues (2008) investigated a number of epidemiological factors in an Australian and New Zealand adult cohort and also reported that males accounted for most TBIs (74%), and that approximately 45% of TBI patients were aged 20-39, however, given their age brackets were broader than Fortune and Wen, and did not include paediatric cohorts, no direct comparison can be made. In Myburgh et al's study severity of TBI was also assessed using the GCS, with more than half of the cohort (57.2%) classified as having sustained a severe TBI, with 18.1% and 24.7% categorised as moderate and mild, respectively. It was noted that the most common cause of TBI was motor vehicle accidents (61.4%), falls (24.9%) and assault (7.2%). However, this study recruited those admitted to intensive care units, or who died in the emergency department or during surgery, thus those with less severe TBIs may not be adequately accounted for.

Butterworth, Anstey, Jorm, and Rodgers (2004) estimated that approximately 6% of adults in Australia had sustained a TBI. Incorporating this finding into their meta-analysis, Frost, Farrer, Primosch, and Hedges (2013) analysed data from The United States of America, Canada, Australia and New Zealand and estimated that approximately 12% of the adult population in these countries had experienced a TBI, with males being 2.2 times more likely than females to sustain a TBI. Although there is a clear difference between these estimations, it should be noted that Butterworth and colleagues required participants to have experienced at least 15 minutes of LOC, whereas Frost and colleagues do not appear to have a minimum length of time for LOC, and therefore the higher prevalence rate is reported in the larger study by Frost and colleagues is unsurprising.

Due to demographic and diagnostic changes, there have been some recent changes in the incidence rates of TBI (Roozenbeek et al., 2013). For example, whilst the incidence rate of TBI in elderly populations has not changed dramatically, with an increasingly aging population as well as the demographic "baby-boomer" bubble there has been a commensurate

increase of TBIs, as reported in international and Australian studies (Myburgh et al., 2008; Roozenbeek et al., 2013). Furthermore, with the development of more sophisticated neuropathological assessment and treatments, there has been some reduction of mortality associated with TBI, although some authors report little change as compared to a decade ago (Myburgh et al., 2008; Roozenbeek et al., 2013). When considered together, these factors suggest that there is likely to be an increase in the number of individuals with TBI, with potentially a larger proportion for whom impairment is significant.

Neuropathology in TBI

From the moment of impact, a series of cerebral events occur which contribute to the short and long term outcomes of TBI. There are two injury processes – primary and secondary. Primary injury effects are a result of the mechanical and compressive forces inflicted by the impact (whether it was caused by a physical object, acceleration/deceleration or percussive force). Secondary injury effects are the neurochemical and physiological changes to the brain triggered by the primary injury effects. While the primary injury processes are immediate and can be catastrophic, the secondary injury processes typically are longer in duration, and have been argued to be more influential in determining long-term outcome (Greve & Zink, 2009).

Primary injury response.

Primary injury describes the immediate mechanical effects of the impact/force of initial injury. Because of the viscoelastic nature of the brain, when there is a blow to the skull, or sudden changes in speed and/or direction of movement, mechanical forces can have

a dramatic effect on brain tissues, with strain and tearing of cells and blood vessels often causing widespread and severe damage (Mustafa & Alshboul, 2013). Furthermore, the brain can rebound against the skull, causing serious trauma due to the skull's inflexibility and bony prominences. There are two forces which exert an effect on the brain structure during impact; linear acceleration and rotation (Greve & Zink, 2009). Linear acceleration damage tends to occur in the cortical surface are area, whereas rotational forces occur within the deeper sub-cortical regions, although in higher velocity damage rotational forces can also cause damage to cortical regions (Greve & Zink, 2009; Post & Hoshizaki, 2012). Although each of these forces is associated with different effects, they rarely, if ever, occur independently of each other in TBI.

According to Post and Hoshizaki (2012), there are four key processes associated with primary injury. Firstly, where there is a direct blow to the head, contusions can occur at the site of impact (referred to as a coup injury), and/or in the opposing brain region, if the brain reverberates from the initial impact and compresses against the opposite part of the skull (contrecoup injury), and damage from impact can also spread laterally. The flexion of the skull bones may also cause damage to the underlying dura (or the vessels that transverse the dura) potentially leading to subdural or extradural haematomas, although the latter are not common, occurring in less than 1% of TBIs (Mustafa & Alshboul, 2013). Tearing of blood vessels within the brain can also cause haematoma within the brain itself (Mustafa & Alshboul, 2013). Often associated with primary injury is secondary damage caused by changes in intracranial pressure (ICP), which can compress and stress brain tissue throughout the brain, or at focal sites. ICP, which can be considered an aspect of both primary and secondary injury, can occur as a result of haematoma and oedema. Furthermore, when the brain changes position relative to the skull, there can be a decrease in ICP in the regions of the brain distal to point of impact, thus a varying pressure gradient may occur and place strain

on the brain tissue throughout the brain (Greve & Zink, 2009; Post & Hoshizaki, 2012). Thirdly, when rotational forces occur, these can inflict damage throughout the brain, and indeed is thought to cause the greatest level of strain on brain tissues (Zhang, Yoganandan, Pintar, & Gennarelli, 2006). Rotational forces are believed to be the primary cause of diffuse axonal injury (DAI), in which axons are torn and stretched, often leading to the cascade of neurological events associated with secondary injury (Mustafa & Alshboul, 2013; Smith, Meaney, & Shull, 2003). Finally, it has been argued that the combination of linear and rotational forces work synergistically, each force exacerbating the effect of the other (Post & Hoshizaki, 2012).

Secondary injury response.

The damage inflicted during primary injury is instantaneous and potentially fatal. However primary injury also triggers a secondary injury response which involves a cascade of biochemical and neurological events, the effects of which can be insidious and long lasting. Where the initial TBI is survived, secondary injury effects are generally considered to be more harmful than primary effects. While secondary injury effects are also more treatable, to date effective treatments have remained elusive (Greve & Zink, 2009; Park, Bell, & Baker, 2008). Secondary injury responses can be broadly divided into two categories; excitotoxic and inflammatory, although it must be noted that these processes are interlinked.

The excitotoxic response to injury typically involves widespread depolarisation across various cell types, and a surge in nitric oxide and excitatory amino acids, most notably glutamate and aspartate (Greve & Zink, 2009). Increases in extracellular glutamate leads to increases in calcium within cells, which is associated with oxidative stress and mitochondrial dysfunction (Walker & Tesco, 2013). There is also an increase in free radicals, and it is believed that these molecules, in conjunction with the increased calcium, trigger further

release of nitric oxide and excitatory amino acids, thus creating an excitotoxic cycle which may spread from injury site to adjacent brain regions, leading to widespread dysfunction and damage (Greve & Zink, 2009).

Inflammatory responses also commence soon after injury, and persist for some time following injury, with some studies finding inflammatory effects lasting years (Johnson et al., 2013; Smith et al., 2013). Neuroinflammation involves increased activation of glial and macrophage cells, and a dramatic change in the expression of a large number of neurochemicals, including pro and anti-inflammatory cytokines and chemokines, (Finnie, 2013; Walker & Tesco, 2013). While some neuroinflammatory responses are beneficial, many processes have been found to be detrimental, and it remains unclear which factors exert positive or negative effects. Indeed, it has been suggested that many factors have a dual role which is possibly mediated by timing of release and levels of production of a given neurochemical (Finnie, 2013; Schmidt, Infanger, Heyde, Ertel, & Stahel, 2004). As for excitotoxic responses, inflammatory responses can also spread to adjacent regions, resulting in more far-reaching damage to tissue (Jaerve & Muller, 2012).

The excitotoxic and inflammatory responses, along with the mechanical forces incurred during TBI, work synergistically leading to a series of harmful pathophysiological changes. The integrity of the blood-brain barrier is often compromised, with increased permeability and reduced capacity to regulate cerebral blood flow (CBF) and other homeostatic mechanisms (Finnie, 2013; Mustafa & Alshboul, 2013; Werner & Engelhard, 2007). CBF is commonly reduced, and can result in focal or widespread ischemia, which is particularly troublesome given the evidence that damaged tissue can be more susceptible to ischemia than healthy brain tissue (Botteri, Bandera, Minelli, & Latronico, 2008). Hypotension may also occur, and there is typically reduced blood flow (hypoperfusion) either focally or globally. Alternatively, individuals can also experience increased CBF, resulting

in hyperperfusion, which can occur in response to injury or due to medical intervention (Werner & Engelhard, 2007). There can also be impaired cerebrovascular autoregulation, resulting in inability to maintain appropriate CBF, and cerebral vasospasm and metabolic dysfunction can also occur. These factors all typically result in hypoxia which is a strong predictor for mortality and morbidity (Greve & Zink, 2009). Oedema also occurs, which involves either an increase in intracellular fluid (cytoxic oedema) or increased extracellular fluid (vasogenic oedema). Typically both types of oedema are present, and the length and timing of both vary depending on the nature of injury, although cytotoxic oedema may be more common than vasogenic oedema (Finnie, 2013; Werner & Engelhard, 2007). Increased cranial pressure (ICP) frequently occurs, due to haemorrhage (caused either by initial injury or by secondary processes) and oedema, and this is argued to be the most harmful secondary process (Finnie, 2013; Greve & Zink, 2009). As a result of these factors, cell death, both necrotic and apoptotic, occurs from time of injury and may lead to increased neuronal degeneration indefinitely (Bigler, 2013; Stoica & Faden, 2010; Walker & Tesco, 2013), and there is evidence that other detrimental events such as increased amyloid pathology occur after TBI (Chen et al., 2004; Marklund et al., 2014).

As can be seen from the above description, the processes that occur as a result of TBI are both complex and long-lasting. Furthermore, there is increasing evidence that there are a number of biological and physiological factors which may exacerbate or ameliorate the progression of injury and subsequent outcomes. The factor of interest in the current work is the APOE gene, which synthesises the protein apolipoprotein E (apoE). As will be demonstrated in the following sections, isoformic differences in apoE have been shown to influence a number of injury processes.

The Role of APOE in TBI

The focus of the current thesis is on the impact of the APOE gene, which synthesises apolipoprotein E (apoE). One of the primary functions of apoE is lipid transport; indeed it is considered the main lipid transporter within the CNS (Leoni, Solomon, & Kivipelto, 2010; Mahley & Huang, 1999). In addition to a transportation function, apoE is implicated in lipid clearance and recycling (Ignatius et al., 1986; Leoni et al., 2010; Struble, Nathan, Cady, Cheng, & McAsey, 2007). Lipids, particularly cholesterol, are essential for normal neurological function and pivotal in the CNS response to TBI (Proust-Lima, Dartigues, & Jacqmin-Gadda, 2011). Being the primary lipid transporter, apoE plays a crucial role in maintenance of neurological integrity and facilitation of post injury repair. There is also evidence that apoE is involved in amyloid accumulation and processing, although the relationship between apoE and amyloid pathology is complex and not yet well understood (Kanekiyo, Xu, & Bu, 2014).

There is strong evidence that apoE is a key factor in the brain's response to injury. Levels of apoE increase dramatically following TBI, with increased levels being reported up to 90 days post injury in rodent studies (Boyles, Pitas, Wilson, Mahley, & Taylor, 1985; Horsburgh, McColl, White, & McCulloch, 2003; Iwata, Browne, Chen, Yuguchi, & Smith, 2005; Proust-Lima et al., 2011; White et al., 2001). This increase appears to occur in both intra and extracellular areas, and both proximal and distal to the injury site (Orihara & Nakasono, 2002). Poirier, Hess, May and Finch (1991) observed that in rats, apoE levels peaked at six days post injury; a time when regeneration of synapses also occurs, suggesting that apoE may play a role in reactive synaptogenesis. Given these results, it has been proposed that apoE promotes sprouting and synaptogenesis in the injured brain, both acutely and in the long term (Orihara & Nakasono, 2002; Snipes, Mcguire, Norden, & Freeman, 1986; White et al., 2001).

Evidence suggests that apoE also has antioxidant properties, and as such it may reduce damage caused by lipid peroxidation and oxidative stress in TBI (Lomnitski et al., 1999; Lomnitski et al., 1997). Lynch, Morgan, Mance and Laskowitz (2001) report that apoE deficiency results in increased inflammatory responses in mixed glial cultures (Lynch et al., 2001), and further research indicated animals deficient in apoE experienced increased oxidative stress, oedema, and more widespread damage following TBI, particularly in hippocampal regions (Han & Chung, 2000; Lynch et al., 2002). Animal studies also indicate that apoE may be important in maintaining the integrity of the blood brain barrier following TBI (Methia et al., 2001), and may influence amyloid aggregation after injury (Hartman et al., 2002; Leclercq, Murray, Smith, Graham, Nicoll, & Gentleman, 2005). Given these functions, it is believed that apoE plays a key role in multiple processes implicated in recovery following TBI (Houlden & Greenwood, 2006; Nicoll & Graham, 1997). Figure 1 depicts the primary and secondary responses associated with TBI, and identifies the processes which have been found to be impacted by the apoE protein.

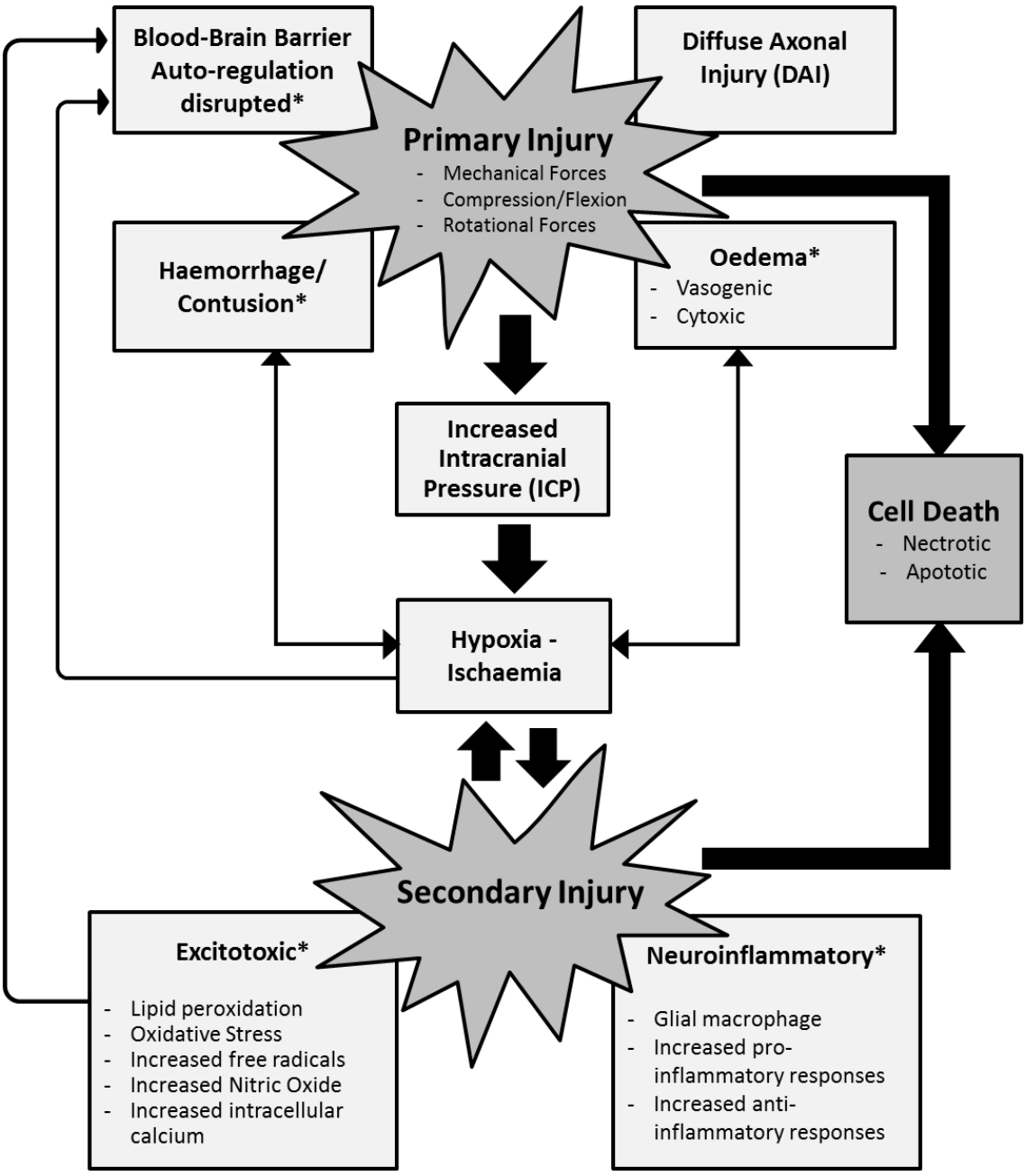

Figure 1. Primary and secondary injury responses (responses reported to be influenced by APOE are asterisked).

APOE genotype: Implications for TBI recovery.

In humans, there are the three common APOE alleles; ε2, ε3, and ε4, with estimated population frequencies of 11%, 72%, and 17% respectively (Zannis, Just, & Breslow, 1981). Each of the three alleles has been found to differ at only two sites – residues 112 and 158.

APOE ε2 has the amino acid cysteine at both residues, APOE ε3 has cysteine at residue 112 and the amino acid arginine at residue 158, and APOE ε4 has arginine at both residues (Rall, Weisgraber, & Mahley, 1982). Despite this relatively small difference, there are noteworthy isoformic differences in the structure of the resultant protein, as well as significant variations in affinity to cellular lipoprotein receptors, and binding to lipids (for a review see Mahley & Rall, 2000). The structure of apoE ε4 has been demonstrated to be the least stable of the three isoforms and has increased misfolding, with evidence that this lack of structural integrity has a negative effect on secondary injury processes (Mahley & Huang, 2006). Notably, APOE ε4 has been found to be associated with greater post-TBI inflammation (Lynch et al., 2003) and also is associated with higher levels of amyloid plaques following TBI (Hartman et al., 2002; Nicholl, Roberts, & Graham, 1995), even when injury is mild (Yang et al., 2015). APOE ε4 has also been found to increase levels of aspartate and histamine post injury (Kerr et al., 2003; Mace et al., 2007), and to also be associated with increased ischaemic damage (Smith, Graham, Murray, Stewart, & Nicoll, 2006). In short, it appears that possession of the APOE ε4 allele is associated with a range of detrimental neuropathological responses following TBI.

APOE ε4 and cognitive function following TBI.

It has been recognised that impaired cognitive ability is the most common complaint following TBI, and that this impairment is strongly associated with functional outcomes, regardless of injury severity (Gronwall & Wrightson, 1974; Konrad et al., 2011; Levin & Robertson, 2013; Spitz, Ponsford, Rudzki, & Maller, 2012). Despite the relatively high incidence of TBI, injury severity estimates such as GCS and PTA have been found to offer limited prognostic value in terms of cognitive impairment, both in short and long term recovery (Azouvi, 2000; Balestreri et al., 2004; Lingsma, Roozenbeek, Steyerberg, Murray,

& Maas, 2010; de Oliveira Thais et al., 2014; Lovell, Iverson, Collins, McKeag, & Maroon, 1999).

In response, there has been growing interest in identifying biological markers that might improve the ability to predict cognitive outcome after TBI, including APOE. Interest in the APOE gene was initially prompted by the reported link between possession of the APOE ε4 allele and increased risk of Alzheimer's disease (Corder et al., 1993; Saunders et al., 1993) and other neurological disorders in which cognitive dysfunction is a defining feature (Alfieri et al., 2008; Borroni et al., 2006; Chang et al., 2011). Despite the evidence that APOE ε4 may have detrimental properties in relation to post-injury neuropathology, how this translates to cognitive function following TBI remains unclear. A number of studies report that APOE ε4 is associated with poorer outcomes post-TBI, but an equal number of studies have reported no effect. Furthermore, there is tentative evidence that APOE ε4 can be ameliorative in some cohorts. The following section reviews the literature to date in which the effect of APOE ε4 on post-TBI cognitive impairment has been investigated, in animal, paediatric, and adult TBI, with a focus on the extant adult TBI literature.

Evidence from animal TBI models.

Although animal models of TBI have demonstrated that a general apoE deficiency reduces cognitive function after injury (Chen, Lomnitski, Michaelson, & Shohami, 1997; Lomnitski, Chen, Shohami, Kohen, & Michaelson, 1997), the evidence regarding the specific effect of APOE ε4 on cognition post TBI is scant, with only two published findings to date. Mannix and colleagues (2011) found that compared to adult wild-type mice, adult APOE ε4 mice displayed impaired cognitive performance following TBI, an effect that remained at up to 12 months post injury, however there was no difference in cognitive function between juvenile APOE ε4 and juvenile wild-type mice. A later study by the same group (Mannix et

al., 2013) found that when repeated mild brain injury occurred APOE ε4 was not predictive of short or long term cognitive impairment, suggesting that any detrimental effects of APOE ε4 may only be present where pathology is moderate to severe. Thus, both reports indicate that although APOE ε4 may have some impact on cognitive function post injury, age and injury severity may moderate outcomes.

Evidence from paediatric TBI cohorts.

Evidence from paediatric cohorts is also limited, with only two studies investigating paediatric TBI using neuropsychological measures. Blackman, Worley and Strittmatter (2005) compared function in 71 children (mean age of 13.16 years) using the Functional Improvement Measure for Children, which provides a discrete measure of cognitive function, at time of injury and again at discharge. They reported that the APOE ε4 carriers (n = 4) displayed better levels of function at time of discharge, suggesting greater recovery than non-carriers. Similarly, Moran and colleagues (2009) used a range of tasks to assess cognitive outcomes at 3 and 12 months following mild TBI in 99 children aged 8 to 15 years old. It was found that APOE ε4 carriers (n = 28) performed better than non-carriers on a visual-motor integration task, with no group differences evident on other tasks. These findings suggest that APOE ε4 is not deleterious, and may confer an advantage in paediatric cohorts, thus aligning with the study by Mannix and colleagues (2011). Conversely, it must be acknowledged that studies assessing general outcome using the extended Glasgow Outcome Scale (GOS-E) after TBI report that paediatric APOE ε4 carriers display poorer outcomes (Kurowski, Martin, & Wade, 2012). However, the GOS-E is not a sensitive neuropsychological measure and there is some question as to the appropriateness of using this scale in paediatric cohorts (Liehlai et al., 1992).

Evidence from adult TBI cohorts.

While research exploring the effect of APOE ε4 in adult TBI cohorts is more frequent, the findings remain equivocal and contradictory. The following two tables provide a condensed summary of the literature to date, and are followed by a more integrative description of the key findings. Table 1 summarises the evidence that indicates APOE ε4 has a detrimental effect post-TBI in adult cohorts, and Table 2 summarises research that indicates APOE ε4 has no effect, or may provide a beneficial effect in adults. In order to further clarify the relationship between APOE status and cognitive function effect sizes are also included for adult TBI studies. Where an effect size has been provided by the authors, these are reported, and where effect size has not been reported, either Cohen's *d* or *r* has been calculated, depending on the nature of the data (for formulae see Dunlap, Cortina, Vaslow, & Burke, 1996; Rosenthal & Rubin, 2003). Magnitude of effect is also estimated, and findings deemed to be clinically relevant have been identified, based on Ferguson's (2009) guidelines for interpreting effect size for the social sciences.

Table 1

Neuropsychological evidence for the detrimental effect of APOE ε4 allele on cognitive outcome following TBI

Author (year)	N	APOE ε4 carrier n	Injury severity	Time of assessment	Cognitive function	Summary of relevant findings	Effect size (magnitude)
Anderson et al. (2009)	51	15	Mild to severe	1, 6, 12, months & 10 years	▪ Verbal memory ▪ Visual-Spatial ability ▪ Psychomotor Speed ▪ Verbal ability	▪ Once adjusted for education APOE ε4 carriers performed significantly poorer in tests of verbal IQ at 1 and 12 months after TBI, and verbal recall at 1, 6, and 12 months after TBI.	d = 0.01 - 0.73 (d = 0.51 [a] -0.73 [a] for significant tests) (Small)
Ariza et al. (2006)	77	10	Moderate to severe	6-9 months	▪ Verbal memory ▪ Visual memory ▪ Executive function ▪ Psychomotor speed	▪ APOE ε4 carriers significantly poorer on 6/9 measures of verbal memory, executive function, and psychomotor speed.	d = 0.27-0.77 (0.50 [a] - 0.77[a] for significant tests) (Small)
Crawford et al. (2002)	110	30	Mild to severe	Within 6 months	▪ Verbal learning and memory ▪ Executive function	▪ APOE ε4 carriers performed worse on verbal memory and learning measures ▪ No significant differences in episodic memory or executive function tasks.	R^2 = .045[a] (Small)
Eramudug-olla et al. (2014)	489	unknown	Mild to severe	Long term	▪ Verbal learning and memory ▪ Verbal ability ▪ Attention ▪ Working memory ▪ Psychomotor speed	▪ TBI sufferers in early adulthood (approx. 20 years old) with APOE ε4 performed worse on tests of episodic memory than non-carriers ▪ Middle-aged TBI sufferers with APOE ε4 (approx. 40 years old) performed poorer than non-carriers of the same age on reaction time tasks, but only when childhood TBI had occurred. ▪ No differences in the cognitive performance in the older age group (approx. 60 years old).	Insufficient data to estimate

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Author (year)	N	APOE ε4 carrier n	Injury severity	Time of assessment	Cognitive function	Summary of relevant findings	Effect size (magnitude)
Friedman et al. (1999)	69	27	Mild to severe	6 - 8 months	▪ General ability	▪ Possession of APOE ε4 was predictive of sub-optimal outcome. A higher percentage of APOE ε4 carriers displayed severe behavioural and cognitive abnormalities, however these didn't reach significance. ▪ No significant interaction between age and APOE.	Behaviour abnormalities OR = 2.71 Severe cognitive abnormalities OR = 2.79[a] (Small)
Müller et al. (2009)	37	13	Mild	Baseline (described as before discharge) and at 6 months	▪ Verbal learning and memory ▪ Working memory ▪ Executive function ▪ Attention	▪ All tasks were combined to determine a global cognitive impairment rating. APOE did not predict initial neuropsychological impairment, but APOE ε4 carriers were slower to recover, and at 6 month follow-up APOE ε4 carriers showed approximately half the improvement of the non- ε4 group.	*d* = .45 (Small)
Noé et al. (2010)	126	23	Moderate to severe	Week after emergence from PTA then at 6 months	▪ Verbal learning and memory ▪ Working memory	▪ APOE ε4 carriers were initially more impaired but improved faster on some working memory tasks. ▪ Authors suggested APOE genotype effects speed of recovery but not amount of recovery.	*d* = 0.10 – 1.25[a] (Small – Med)

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Author (year)	N	APOE ε4 carrier n	Injury severity	Time of assessment	Cognitive function	Summary of relevant findings	Effect size (magnitude)
Sundström et al. (2004)	30	11	Mild	Within 5 years	▪ Attention ▪ Verbal learning and memory ▪ Visual learning and memory ▪ Visual-Spatial ability ▪ Executive function	▪ Comparison of pre and post injury cognitive performance revealed that APOE ε4 carriers had a significant post-injury decline in three out of nine tasks (divided attention, recognition and recall), whereas there were no significant declines in the non- ε4 carriers.	*d* = 0.03 - 0.78 Divided attention *d* = .78[a] Recognition *d* = .67[a] Recall *d* = .36 (Small)

[a] = Effect size is considered to be clinically significant, as recommended by Ferguson (2009)

OR = Odds Ratio

Table 2

Neuropsychological evidence of no effect of APOE ε4 allele on cognitive outcome following TBI

Author (year)	N	APOE ε4 carrier n	Injury severity	Time of assessment	Cognitive function	Summary of relevant findings	Effect size (magnitude)
Ashman et al. (2008)	54	15	Mild to severe	1-58 years (M = 12.8 years)	▪ Verbal memory and learning ▪ Visual memory and learning ▪ Processing Speed ▪ Executive Function ▪ Attention ▪ Verbal ability/fluency	▪ No significant differences in cognitive decline based on APOE status.	Insufficient data to estimate
Chamelian et al. (2004)	90	19	Mild	6 months	▪ Working memory ▪ Verbal memory and learning ▪ Visual memory and learning ▪ Processing Speed ▪ Attention ▪ Executive function	▪ No significant differences for any measures.	d = 0.11 - 0.40 (Small)
Han et al. (2007)	78	16	Mild to moderate	1 month	▪ Working memory ▪ Processing Speed ▪ Attention ▪ Visual-Spatial ability ▪ Executive function ▪ Verbal memory and learning	▪ APOE ε4 carriers performed better than non-APOE ε4 carriers on some measures of working memory and verbal learning, with trends for better performance on a number of other executive function, working memory and verbal learning measures.	η_p^2 = 0.00 - 0.14[ab] (Small)

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Author (year)	N	APOE ε4 carrier n	Injury severity	Time of assessment	Cognitive function	Summary of relevant findings	Effect size (magnitude)
Hodgkinson et al. (2009)	58	13	Severe	12 months	▪ Verbal memory and learning ▪ Visual memory and learning ▪ Attention ▪ Working memory ▪ Visual-spatial ability	▪ No significant differences between APOE ε4 and non- ε4 carriers on any tasks except DS, where APOE ε4 carriers performed better than non- ε4 carriers.	*d* = 0.03 - 0.80 [ab] (Small)
Isoniemi et al. (2006)	61	19	Mild to severe	Approx. 30 years	▪ Verbal memory and learning ▪ Visual memory and learning ▪ Verbal fluency/ability ▪ General ability	▪ Although initial analysis revealed APOE ε4 carriers performed significantly worse than non APOE ε4 carriers, this result was attributed to six individuals who had developed dementia related symptomology.	*d* = .04 – 1.00[a] (Small)
Liberman et al. (2002)	80	18	Mostly mild	3 and 6 weeks	▪ Psychomotor speed ▪ Attention ▪ Processing speed ▪ Executive function ▪ Verbal memory and learning ▪ Visual memory and learning	▪ After controlling for age, sex, education and ethnicity, APOE ε4 carriers were found to perform poorer on 12 out of 13 tasks, as compared to non- ε4 carriers, however only two tasks reached significance (PASAT and grooved pegboard). A similar finding was reported for the second visit, where APOE ε4 carriers performed worse on 11 out 13 tasks for visit 2, however none were significant. ▪ Authors suggest presence of APOE ε4 may impact on initial severity of impairment, but not rate of recovery.	*d* = 0.004 - 0.61 (0.59[a] and 0.61[a] for significant tasks) (Small)

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Author (year)	N	APOE ε4 carrier n	Injury severity	Time of assessment	Cognitive function	Summary of relevant findings	Effect size (magnitude)
Lichtman et al. (2000)	31	7	Mild to Severe	6 months	▪ General ability	▪ Significant differences between APOE ε4 and non- ε4 carriers for FIM total and motor subscale, but not FIM cognitive subscale	FIM Total *d* = 0.5[a] Cognitive Subscale *d* = 0.2 (Small)
Millar et al. (2003)	396	117	Mild to severe	15-25 years	▪ Verbal memory and learning ▪ Visual memory and learning ▪ Attention ▪ Verbal ability/fluency ▪ Executive function	▪ APOE ε4 status was not predictive of overall cognitive function, or for any of the specific cognitive domains. ▪ APOE ε4 carriers who were initially classified with severe disability at time of injury demonstrated significantly poorer cognitive function than non-APOE ε4 carriers who were also rated as being severely disabled.	OR 0.83 – 1.54 (Small)
Ponsford et al. (2007)	120	28	Moderate to severe	3, 6, and 12 months	▪ Verbal memory and learning ▪ Attention ▪ Executive function	▪ No significant differences due to APOE status on any tasks at any time point with the exception of the SMDT at 3 months post injury.	SMDT *r* = .20[a] (Small)
Pruthi et al. (2010)	73	12	Mild to moderate	6 months	▪ Attention ▪ Visual learning and memory ▪ Psychomotor speed ▪ Verbal ability	▪ No significant differences between APOE ε4 and non- ε4 carriers on any tasks.	Insufficient data to estimate
Rapoport et al. (2008)	69	Unknown	Mild to moderate	12 and 24 months	▪ Working memory ▪ Verbal learning and memory ▪ Visual learning and memory ▪ Executive function	▪ No significant differences between APOE ε4 and non- ε4 carriers on performance on neuropsychological tasks, or clinician assessment of cognitive impairment.	Insufficient data to estimate cognitive tasks. Clinician assessment *d* = 0.37 (Small)

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Author (year)	N	APOE ε4 carrier n	Injury severity	Time of assessment	Cognitive function	Summary of relevant findings	Effect size (magnitude)
Shadli et al. (2011)	19	6	Mild to Moderate	6 weeks and 6 months	▪ Visual-spatial ability ▪ Verbal learning and memory ▪ Executive function	▪ APOE ε4 carriers did not perform worse than non-carriers who sustained TBI on any neuropsychological tasks.	η^2 = .002 - .087[a] (Small)
Teasdale et al. (2000)	39	10	Severe	Approx 1.9 years post injury and 12 months later	▪ Attention ▪ Verbal memory and learning ▪ Visual memory and learning	▪ Comparison of cognitive function at the commencement and cessation of a rehabilitation programme revealed that performance was the same for both APOE ε4 carriers and non-carriers, with the exception that APOE ε4 carriers had greater improvement on one memory task.	*d* = 0.00 – 0.85[ab] (Small)

a = Effect size is considered sufficient to be of clinical significance, as recommended by Ferguson (2009)

[b] = APOE ε4 carriers performed significantly better than APOE non-ε4 carriers on at least one task

LR = Likelihood ratio

OR = Odds Ratio

η^2 = Eta Square

η_p^2 = Partial Eta Square

Although the literature regarding the effect of APOE ε4 on cognitive function after TBI is mixed, the above findings do offer some insights. Firstly, the effect sizes reported above demonstrate that the magnitudes of effect are consistently small. Nonetheless, many of these findings would still be considered clinically relevant based on the guidelines provided by Ferguson (2009). Secondly, significant differences are most frequently reported in the first 12 months following injury, with a number of authors observing that APOE ε4 carriers displayer poorer cognitive performance in this period. The following sections describes the findings relating to recovery in the first 12 months after TBI, and this is followed by a summary of research relating to later outcomes.

APOE ε4 and cognitive function in the first 12 months following TBI in adults.

A number of researchers have reported that APOE ε4 is associated with either greater cognitive impairment, or reduced recovery trajectories in the 12 months following TBI. Crawford and colleagues (2002) reported that verbal memory was significantly more impaired in APOE ε4 carriers than in non-carriers at 6 months following TBI, however there were no significant differences in measures of executive function. Similarly, Ariza and colleagues (2006) found that APOE ε4 carriers performed poorer on tasks of verbal memory, as well as psychomotor speed, visual tracking and attention, and executive function, at 6 to 9 months post injury. It is noteworthy that in the study by Ariza et al there were no significant differences on the GOS-E despite poorer performance on a large number of neuropsychological tasks, suggesting that general measures of function such as the GOS-E are not sufficiently sensitive to detect cognitive impairment. Friedman et al (1999) also assessed cognitive function at 6-8 months following TBI, and reported that APOE ε4 carriers had more serious behavioural and cognitive abnormalities, although it in not clear which cognitive domains were evaluated.

Although the above findings indicate APOE ε4 carriers may have greater impairment than non-carriers during the first year after TBI , a number of investigations have failed to find any significant differences in the same time period (Table 2; Chamelian, Reis, & Feinstein, 2004; Hodgkinson, Gillett, & Simpson, 2009; Liberman, Stewart, Wesnes, & Troncoso, 2002; Lichtman, Seliger, Tycko, & Marder, 2000; Pruthi et al., 2010; Shadli, Pieter, Yaacob, & Rashid, 2011), although it is noteworthy that when Liberman et al compared the performance of APOE ε4 carriers and non-carriers on a battery of 13 neuropsychological tests 3 and 6 weeks after TBI, they observed that, although not reaching significance, the APOE ε4 group displayed poorer performances than the non-carrier group on all but one task at the first visit, and all but two tasks at the second visit. The consistency in direction of effects suggests that a larger sample size may have revealed significant differences.

There is also some evidence that possession of APOE ε4 might lead to reduced recovery trajectories. Müller and colleagues (2009) compared APOE ε4 carriers and non-carriers cognitive performance at time of injury and at 6 months following mild TBI and reported that although there were no differences between APOE ε4 and non-carriers at the baseline assessment, APOE ε4 carriers displayed a lower global score at 6 months. It was reported that non-carriers had approximately twice the rate of recovery of the APOE ε4 group, indicating a significantly slower recovery trajectory associated with APOE ε4. The authors also observed that APOE genotype was a better predictor of 6 month outcome than Glasgow Coma Scale score (GCS) or other neurological indicators.

Interestingly, others have reported the opposite effect. Despite using similar tasks to Müller et al., Noé, Ferri, Colomer, Moliner and Chirivella (2010) found that APOE ε4 carriers initially displayed greater cognitive impairment than non-carriers, but then recovered at a faster rate, and by 6 months there were no significant differences between the two

groups. While this finding appears to be at odds with Müller et al (2009), there are two distinctions that should be noted. Firstly, Müller et al recruited participants with mild TBI (classified as 13-15 on the GCS), whereas the participants in Noé et al's study had moderate to severe TBI (GCS < 12). Therefore it is possible that the effect of APOE ε4 is accentuated by other factors associated with more severe neuropathology during early recovery; a finding which aligns with the previously described animal study by Mannix et al (2013), but does not explain the faster recovery rate reported by Noé. Secondly, in Noé et al's study the differences were only apparent in the some of the working memory tasks; the APOE ε4 group did not display superior rates of recovery across the majority of tasks. Given that Müller and colleagues used a global score, it is difficult to compare these two findings in terms of whether similar domains were affected.

Anderson and colleagues (2009) also employed a longitudinal design when they assessed neuropsychological performance at 1, 6, and 12 months, and 10 years post-TBI. When education was controlled for, APOE ε4 carriers performed significantly worse than non-carriers on tasks of verbal memory at 1, 6 and 12 months following injury, and on the verbal intelligence quotient subscale of the Wechsler Adult Intelligence Scale at 1 and 12 months post TBI. No significant differences were found on any tasks at the 10 year time-point, suggesting that possession of the APOE ε4 allele may be associated with delayed recovery for a period of time following injury, but not necessarily progress into chronic impairment. This supports the findings reported in the shorter-term studies (Ariza et al., 2006; Crawford et al., 2002; Friedman et al., 2009) described above. In contrast, Ponsford, Rudzki, Bailey and Ng (2007) failed to find any differences using similar tasks at 3, 6 and 12 months post-injury, with the exception of a single attention task at 3 months. It is difficult to reconcile these conflicting findings given the sample characteristics and measures used in both studies are relatively similar.

While the majority of published adult TBI studies indicate that APOE ε4 carriers either have poorer cognitive outcomes or no differences in cognitive outcomes to non- ε4 carriers, Han et al (2007) report contradictory findings. In an examination of the neuropsychological performance of young adults within a military population 1 month following a mild to moderate TBI, Han et al (2007) found that APOE ε4 carriers performed significantly better than non-carriers on several measures of working memory, verbal learning and recall, and executive function. Although this finding implies APOE ε4 may have a beneficial effect for young adult TBI sufferers, it must be noted that for the majority of tasks, there were no significant differences.

APOE ε4 and long-term cognitive function following TBI in adults.

There is less evidence that APOE ε4 is associated with longer-term cognitive impairment, however, some long-term studies have also reported a detrimental effect of APOE ε4 on cognitive function. Sundström et al (2004) compared pre- and post-injury neuropsychological performance by recruiting through an ongoing longitudinal health study. Individuals who reported sustaining a TBI within the 5 year period between assessments (approx. 19 months after TBI) were grouped according to APOE genotype, and the authors reported greater cognitive decline in the APOE ε4 group following TBI on attention and memory tasks. It should be noted that significant post-injury decline in the APOE ε4 group was only observed on three out of nine measures. In another retrospective study, Eramudugolla et al (2014) compared cognitive decline following self-reported TBI in three adult cohorts (early, middle and old-aged groups – aged approx. 20, 40 and 60 years respectively) who were assessed three times across an 8 year period. When APOE ε4 carriers and non-carriers were compared within each age cohort, it was found that APOE ε4 carriers in early adulthood experienced greater declines in episodic memory than non-carriers, and

that the APOE ε4 carriers in the middle aged cohort had significantly greater declines in reaction time than non-carriers. Those in older adulthood showed no significant differences based on APOE ε4 status. It was also noted that there were significant differences between the sexes, with females performing worse than males in the middle and later adulthood groups, however the interaction between sex and genotype was not investigated. The reliance on self-report of the timing and severity of the TBI reduces the reliability of this finding, as many participants were self-reporting and estimating injury from many years previous. Furthermore, the substantial variation in time of follow-up assessment in both Sundström et al and Eramudugolla et al's studies may also have obscured effects should there be differing recovery trajectories as reported in other literature (Anderson et al., 2009; Noé et al., 2010; Müller et al., 2009).

Alternatively, Teasdale and colleagues (2000) compared APOE ε4 carriers and non-carriers cognitive function on a range of tasks at the commencement of a rehabilitation programme (approximately 1.9 years after injury) and 12 months later. Despite subjective reports by APOE ε4 carriers and their relatives indicating they had poorer cognitive recovery, actual performance on neuropsychological tasks was the same for both APOE ε4 carriers and non-carriers, with the exception that APOE ε4 carriers showed greater improvement on one verbal memory measure; a result the authors thought likely to be spurious.

Very long term studies have found no significant differences based on APOE status following TBI (Anderson et al 2009; Ashman et al, 2008; Isoniemi, Tenovou, Portin, Himanen & Kairisto, 2006; Millar, Nicholl, Thornhill, Murray, & Teasdale, 2003; Rapoport et al, 2008). However, Millar and colleagues observed that the APOE ε4 carriers with severe TBI had poorer cognitive function than non-carriers who had also sustained a severe TBI, suggesting that increasing injury severity may pronounce the effect of APOE ε4. Isoniemi et al also assessed individuals who had sustained a TBI 30 years earlier (age at testing approx.

60 years), and reported that APOE ε4 carriers had significantly poorer cognitive outcomes than non-carriers, although further analysis revealed this was solely due to a subgroup of six individuals who had developed dementia symptoms. These findings are somewhat surprising given the hypothesised relationship between APOE ε4 and AD incidence (Corder et al., 1993); if the relationship between APOE and AD is due to APOE ε4 carriers having increased age-related cognitive decline it would be expected that APOE ε4 carriers in this age group would display greater cognitive decline than non- ε4 carriers, regardless of whether or not a TBI had been sustained. Collectively, the above findings indicate that although the APOE ε4 allele may be associated with delayed recovery, particularly within the first year following injury, this does not appear to progress into chronic impairment.

Issues associated with APOE ε4 research in TBI populations.

Given the mixed findings reported to date, it is suggested that a more nuanced approach to exploring the role of APOE status in the TBI population is needed. For example, there have been calls for more detailed investigations of all three APOE alleles, rather than focus on APOE ε4 (Ponsford, 2013; Weaver et al., 2014). To date, it has been routine practice to categorise participants as either ε4-carriers (possessing a genotype of ε2/ε4, ε3/ε4 or ε4/ε4) or non-carriers (ε2/ε2, ε2/ε3, or ε3/ε3). However, there is emerging evidence that the ε2 allele is dominant over the ε3 and ε4 alleles, and may confer protection following insult or injury (Corder et al., 1994; Laskowitz, Horsburgh, & Roses, 1998; Mahley & Rall 2000; Miller et al., 2010). Thus, inclusion of individuals with an ε2/ε4 genotype may mask any deleterious effects of ε4, and alternately the inclusion of ε2 carriers in the non-carrier cohort may inflate differences between ε4-carriers and non-carriers. It has also been suggested that APOE ε2 carriers should be grouped separately, given the potential protective properties of this allele (Suri, Heise, Trachtenberg, & Mackay, 2013).

It has also been claimed that the expression of APOE may be influenced by biological and environmental factors (Ordovas, 2007). Specifically, there is emerging evidence that sex may interact with APOE ε4 in relation to risk of developing AD (Altman et al., 2014; Ungar, Altmann & Greicius, 2014), and that both age and sex might interact with APOE ε4 in relation to cognitive function (Beydoun et al., 2012; Jack et al., 2015). Despite this, there are yet to be any investigations employing neuropsychologically-sensitive tasks to investigate the relationship between sex and APOE status, and there are very few published studies exploring of the interaction between age and APOE status in TBI populations. The focus of this thesis is therefore to more fully explore the role of APOE ε4 in post-TBI cognitive function by comparing the effects of APOE ε4 to the predominant genotype; APOE ε3, and if possible, also exploring the effect of the APOE ε2 allele. Additionally, the relationship between sex and age and the APOE gene is of interest in the current thesis, and the following section provides a brief review of the evidence regarding these issues.

The effect of age on APOE ε4 expression: Evidence of antagonistic pleiotropy?

It is possible that any detrimental effect of APOE ε4 is moderated by age, and that APOE ε4 may even be beneficial in young individuals. For example, animal studies have found that APOE ε4 may be associated with poorer cognitive performance in both injured and non-injured older adults, but not in juvenile animals (Mannix et al., 2011; Raber et al., 1998; Veinbergs et al., 1999). And, as noted previously, there is evidence that the APOE ε4 allele may be associated with improved cognitive performance in paediatric TBI cohorts (Blackman, Worley, & Strittmatter, 2005; Moran et al., 2009), and in young adult TBI populations (Han et al, 2007). However, in adult TBI populations, there are only two studies that have explored the effect of age; one reporting no age-related interactions (Friedman

1999), and the other reporting a decreased difference in cognitive impairment between APOE ε4 carriers and non-carriers post TBI in later life (Eramudugolla et al, 2014).

The difference between findings in juvenile and young adults, as compared to older cohorts, might be reconciled by considering the concept of antagonistic pleiotropy. The antagonistic pleiotropy hypothesis proposes that gene(s) may exert beneficial effects upon a given trait before and during the reproductive phase of life, and have a deleterious effect in post-reproductive life (Carter & Nguyen, 2011), and some authors have suggested this explains the relationship between APOE polymorphism and Alzheimer's Disease (Han & Bondi, 2008; Leroi et al, 2005; Tuminello & Han, 2011). There is a growing body of evidence that APOE does indeed have an antagonistic pleiotropic effect on a range of non-cognitive traits (Jasienska, Ellison, Galbarczyk, Jasienski, & Kalemba-Drozdz, 2015; Kulminski et al., 2013; Kulminski et al., 2011; Wierenga et al., 2013), and possibly has a similar effect on cognitive function in some clinical cohorts (Chang et al., 2011). However the evidence for an antagonistic effect on cognitive function remains inconclusive, with some finding evidence to support this mechanism (Jochemsen, Muller, van der Graaf, & Geerlings, 2012; Marchant, King, Tabet, & Rusted, 2010; Mondadori et al., 2007; Nichols et al., 2012; Rusted et al., 2013; Yu, Lin, Chen, Hong, & Tsai, 2000), and others finding no effect (Bunce, Anstey, Burns, Christensen, & Easteal, 2011; Bunce et al., 2014; Jorm et al., 2007). The antagonistic pleiotropic hypothesis is yet to be applied to cognitive impairment for APOE ε4 carriers versus non-carriers following TBI, but may prove to be a fruitful line of research given some of the age effects described above.

The effect of sex on APOEε4 expression: Are female APOE ε4 carriers more vulnerable to cognitive impairment?

It has been established that higher levels of oestrogen increase production of apoE (Horsburgh, Macrae, & Carswell, 2002; Struble, Nathan, Cady, Cheng, & McAsey, 2007), with evidence indicating that allelic variations of APOE may influence this relationship (Lambert, Coyle, & Lendon, 2004). There is also tentative evidence to suggest that there are sex differences which may interact with APOE status to influence cognitive function, with Raber and colleagues (1998) reporting that older APOE ε4 carrying female mice displayed poorer cognitive performance than their male counterparts (Raber et al., 1998). Furthermore, in both healthy and cognitively impaired human populations, females who possess APOE ε4 have been found to have greater age related cognitive decline than APOE ε4 males (Bartres-Faz et al., 2002; Fleisher et al., 2005; Mortensen & Hogh, 2001). Other researchers have reported that oestrogen use by post-menopausal females reduces age related cognitive decline in non-carriers, but does not slow cognitive decline in APOE ε4 carriers, suggesting that the interaction between sex and APOE is less efficacious for APOE ε4 carriers (Yaffe, Haan, Byers, Tangen, & Kuller, 2000). Interestingly, Ponsford and colleagues (2011) also reported that following TBI, female APOE ε4 carriers over the age of 55 had poorer outcomes (as measure by the GOS-E) than males of the same age, or than female non-carriers. These findings may indicate that in female APOE ε4 carriers, premenopausal levels of oestrogen ameliorate the detrimental effect of APOE ε4 by increasing apoE levels, but when oestrogen levels drop following menopause, apoE production also declines, and therefore the detrimental effect of the poorly structured apoE ε4 isoform is increased. However this hypothesis is yet to be tested and is thus highly speculative.

Although none of the neuropsychological studies reviewed above investigated potential sex differences in relation to APOE ε4, Eramudugolla and colleagues (2014)

observed in their long term retrospective study that young adult females tended to outperform males of the same age, and older females performed worse than their male counterparts on a number of measures memory and reaction time, however the authors did not investigate the interaction between sex and genotype. In contrast, Alexander and colleagues (2007a) reported no interaction between APOE and sex in their investigation of general functional outcome during the first two years after severe TBI. Given the relationship between sex hormones and APOE described in the above, investigation of the interaction between sex and APOE, using sensitive neuropsychological tasks, rather than functional outcome measures, is needed.

Thesis Aim and Chapter Outline

The aim of the thesis was to provide a more nuanced and rigorous exploration of the effect of APOE ε4 on cognitive function following TBI than has previously occurred. This was done firstly by conducting a meta-analysis of the extant literature, as no such analysis had been published at the time of writing. Based on the conclusions drawn from the meta-analysis, and from the broader review of literature, two studies were undertaken; one during the acute recovery period, and a second longitudinal study at 3, 6 and 12 months after TBI, both of which employed sensitive neuropsychological tasks.

In order to provide a more focused exploration of the impact of APOE ε4 on post-TBI cognitive function, the majority of the research undertaken in this thesis compares ε4 carriers who did not also possess the APOE ε2 allele (thus, those with either an ε4/ε3 or ε4/ε4 genotype) to participants who were homozygous for APOE ε3 allele (those with a homozygous ε3/ε3 genotype), due to the potentially opposing effects of the APOE ε2 and APOE ε4 alleles. Beyond exploring the relationship between APOE status and post-TBI cognitive function, there were additional objectives of investigating the potential interactions

between APOE ε4 and age, APOE ε4 and sex, and APOE ε4 and TBI severity. While it was planned to examine the effect of the APOE ε2 allele, due to the relative population infrequency of this allele it was not certain that sufficient participants with this allele would be identified. Thus, there were five hypotheses:

- **Hypothesis 1:** That APOE ε4 carriers (ε4/ε3 and ε4/ε4) who had sustained a TBI would experience poorer cognitive function than APOE ε3 homozygotes in the first 12 months after injury. This is the primary hypothesis of this thesis and is explored in the studies reported in chapters 2, 5, and 6.

- **Hypothesis 2:** That age would interact with APOE ε4 via an antagonistic pleiotropic mechanism, whereby young adult APOE ε4 carriers (ε4/ε3 and ε4/ε4) would not experience cognitive impairment (or may even display superior performance) as compared to APOE ε3 homozygotes of the same age, whereas older APOE ε4 carriers would demonstrate poorer cognitive function than APOE ε3 homozygotes of the same age, following TBI. This question is addressed in chapter 6.

- **Hypothesis 3:** That there would be an interaction between APOE ε4 and sex, resulting in females being more vulnerable to the effect of APOE ε4 than males, in relation to post-TBI cognitive function. This hypothesis is investigated in chapter 6.

- **Hypothesis 4:** In severe TBI that APOE ε4 carriers (ε4/ε3 and ε4/ε4) would experience more pronounced cognitive impairments than APOE ε3 homozygotes. This hypothesis is explored in chapter 5.

- **Hypothesis 5:** Contingent on sufficient recruitment of ε2 carriers, that APOE ε2 carriers would outperform both APOE ε4 carriers and APOE ε3 homozygotes. This question is addressed in chapter 5.

Chapter 2 contains the meta-analysis of the research to date. This meta-analysis incorporates literature from adult TBI populations in which neuropsychological assessment was undertaken within the first 12 months following injury. As will be demonstrated, despite conducting separate analyses for general cognitive function and domain-specific cognitive function, there was no evidence of APOE ε4 having a detrimental impact on cognitive function. Possible reasons for this finding and directions for future research, are offered. The content of this chapter is currently in press:

Padgett, C. R., Summers, M. J., & Skilbeck, C. E. (in press). Is APOE ε4 associated with poorer cognitive outcome following traumatic brain injury? A meta-analysis. *Neuropsychology.*

Chapters 3 and 4 provide the general method for this thesis. Given the longitudinal nature of the research, it was foreseen that missing data would be likely, and although not directly related to the research questions described above, it was therefore also decided that using the clinical data collected for this thesis would provide a valuable opportunity to explore the effect of missing data on clinical data. Specifically, a series of traditional and more sophisticated statistical approaches used to compensate for the effects of missing data were applied to a sub-set of the data to determine the relative effects of each technique. The findings from this were also published in a peer reviewed journal, which forms the basis of chapter 4:

Padgett, C. R., Skilbeck, C. E., & Summers, M. J. (2014). Missing data: The importance and impact of missing data from clinical research. *Brain Impairment, 15*, 1-9.

Chapter 5 reports the findings on the effect of APOE ε4 on cognitive function during the acute recovery period following TBI once other injury and demographic-factors were accounted for. An advantage of this study over previous studies which have used neuropsychological assessment during the acute recovery phase were the additional analyses in which of APOE ε2 carriers were treated a separate group, and an investigation of the effect of APOE ε4 specifically in moderate to severe TBI. This series of analyses indicated that APOE ε4 was unlikely to influence initial cognitive recovery, even where injury was more severe, and provided tentative evidence that APOE ε2 did not appear to influence recovery. These findings are currently in press:

Padgett, C. R., Summers, M. J., McCormack, G. H., Vickers, J. C. & Skilbeck, C. E (in press). Exploring the effect of the APOE gene on executive function, working memory and processing speed during the early recovery period following traumatic brain injury. *Journal of Clinical and Experimental Neuropsychology.*

Chapter 6 contains the final study for this thesis, in which the interaction between APOE ε4, sex, and age, are investigated in the context of cognitive impairment at 3, 6 and 12 months post-injury. In this study, no interactions were found between APOE ε4 and age or sex, however APOE ε4 carriers performed worse than non-carriers on the Trail-Making Task B (TMTB) at 6 months post-TBI, and there was a trend for poorer performance on the Controlled Oral Word Association Task (COWAT). This may provide evidence for a

domain-specific effect of APOE ε4, whereby APOE ε4 carriers experience poorer executive function, however this finding must be interpreted cautiously unless confirmed by future research. This study has been submitted and is undergoing review:

Padgett, C. R., Summers, M. J., Honan, C. A., McCormack, G. H., Vickers, J. C., & Skilbeck, C. E. (under review). Does Apolipoprotein ε4 interact with age or sex in cognitive function after traumatic brain injury? *Brain [BRAIN-2016-00203]*.

Chapter 7 concludes with a general discussion which summarises the key findings, and describes the limitations and applications of this thesis. The final section of this chapter aims to provide directions for future research.

Chapter 2: Is APOE ε4 Associated with Poorer Cognitive Outcome Following Traumatic Brain Injury? A Meta-analysis.

Published as:

Padgett, C. R., Summers, M. J., & Skilbeck, C. E. (in press). Is APOE ε4 associated with poorer cognitive outcome following traumatic brain injury? A meta-analysis. *Neuropsychology*.

Abstract

Objective: Cognitive impairment is a common sequelae of traumatic brain injury (TBI), however predicting who will experience poorer outcomes remains challenging. A potential risk factor that has gained attention is the APOE gene, with the ε4 allele hypothesised to have a detrimental effect on post-TBI cognitive outcome. The aim of this meta-analysis was to evaluate the effect of APOE ε4 both in terms of general cognitive function and within specific domains known to be prone to impairment following TBI (executive function, working memory, verbal memory and visual memory).

Method: A literature search was undertaken in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA), resulting in the inclusion of ten studies (ε4-carriers n = 143, non-carriers n = 510). Neuropsychological tasks were identified and Cohen's d was calculated and pooled. Meta-analyses were conducted on general cognitive functioning, and for the specific cognitive domains of interest.

Results: No significant differences were found between APOE ε4-carriers or non-carriers, either in general cognitive function, or in the cognitive domains of executive function, working memory, verbal memory or visual memory.

Conclusions: This meta-analysis indicates that APOEε4 does not have a detrimental effect on cognitive performance following TBI. We propose that the relationship between APOE and cognitive function following TBI is complex, and a more nuanced exploration of APOE genotypes is needed.

Keywords: Traumatic Brain Injury, Apolipoprotein E, Cognitive Function, Executive Function, Working Memory, Visual Memory, Verbal Memory.

Introduction

Investigations of candidate gene-environment interactions (cGxE) have become increasingly popular within psychological research. The initial proliferation of significant findings in a broad range of cGxE investigations attracted widespread attention, with recent advances and greater cost-efficiency of genotyping technologies resulting in a burgeoning number of publications in recent years. An emerging area of research is the exploration of gene-related effects following traumatic brain injury (TBI), including the possibility that cognitive impairment may be impacted by the expression of a given gene. Cognitive impairment is considered the most common and enduring complaint following TBI, and is often reported to be the greatest impediment to resumption of normal life (Draper & Ponsford 2008; Konrad et al., 2011; Spitz, Ponsford, Rudzki, & Maller, 2012). The ability to predict who will experience impairment remains poor, with physiological measures of injury severity and pre-morbid characteristics offering limited prognostic value, especially in mild TBI (Azouvi 2000; Jacobs et al., 2013; Lannsjo, Backheden, Johansson, af Geijerstam, & Borg, 2013; de Oliveira Thais et al., 2014). The APOE gene has received attention as a potential predictor of injury severity and outcome, with research focussing on the ε4 allele.

The APOE gene: General characteristics and function.

The APOE gene is located on the long arm of chromosome 19, and expresses the amino-acid glycoprotein Apolipoprotein E (apoE; Scott, Knott, Shaw, & Brook, 1985). ApoE is considered to be the primary lipid transporter within the central nervous system, providing cholesterol and other lipids which are essential for maintaining neurological integrity and facilitation of post injury repair (Mahley, 1988), and is also believed to play a role in lipid clearance and recycling (Ignatius et al. 1986; Leoni, Solomon, & Kivipelto, 2010; Mahley & Huang, 1999). Consistent with this, it has been shown that levels of apoE

increase dramatically in response to TBI (Horsburgh, McColl, White, & McCulloch, 2003; Poirier, Hess, May, & Finch, 1991). ApoE has also been reported to be involved in multiple processes implicated in cellular recovery following TBI, including: maintaining the integrity of the blood brain barrier (Methia et al., 2001); neural growth and regeneration (Horsburgh, Graham, Stewart, & Nicholl, 1999; Mahley & Rall, 2000); reactive synaptogenesis (Poirier et al., 1991); acute and long-term sprouting and synaptogenesis (Orihara & Nakasono 2002; Snipes, Mcguire, Norden, & Freeman, 1986; White, Nicholl, & Horsburgh, 2001); and reduction of oxidative stress and oedema, particularly in hippocampal regions (Han & Chung 2000; Lynch et al., 2002). In short, there is strong evidence to indicate that apoE is a crucial protein that is associated with a wide range of neurobiological responses to TBI.

In humans, there are three common APOE alleles; ε2, ε3, and ε4, with population frequencies of approximately 11%, 72%, and 17% respectively (Eisenberg, Kuzawa, & Hayes, 2010; Zannis, Just, & Breslow, 1981), with each allele synthesising a structurally different form of protein (Utermann, Langenbeck, Beisiegel, & Weber, 1980). The protein synthesised by the APOE ε4 allele has been demonstrated to be the least stable of the three, with evidence of increased misfolding in the resultant protein, which is believed to contribute to neurodegeneration and apoptosis (for a review see Mahley & Huang, 2012). APOE ε4 has been associated with increased incidence and poorer outcomes in Alzheimer's disease and other neuropathological events (Saunders et al., 1993; Strittmatter et al., 1993; Verghese, Castellano, & Holtzman, 2011), particularly when present in the homozygous condition, indicating a possible dose-dependent effect (Corder et al, 1993; Engelborghs et al, 2006; Hostage et al, 2013).

APOE ε4 and cognition.

While there is convincing evidence that APOE ε4 is associated with neurodegenerative disorders in which cognitive decline is a defining feature, there are mixed findings regarding the nature of the relationship between APOE ε4 and cognitive function in other clinical populations. For example, Louko, Vilkki, and Niskakangas (2006) reported that ε4-carriers performed worse than non-carriers on tasks of visual memory, verbal fluency and attention following aneurysmal subarachnoid haemorrhage, and Wagle et al (2010) found that ε4-carriers performed worse than non-carriers on tests of verbal memory and learning following stroke. There is also evidence that APOE ε4 is associated with a more rapid progressive cognitive decline in HIV and Parkinson's disease (Chang et al, 2014; Mata et al, 2014). However, others have found no meaningful differences between the cognitive performance of ε4-carriers and non-carriers in the same clinical populations (Becker et al, 2015; Bour et al, 2010; Federoff, Jimenez-Rolando, & Singleton, 2012; Morris, Wilson, Dunn & Nicholl, 2004).

The relationship between APOE ε4 and cognition is equally contentious in healthy populations. Small et al (2004) and Wisdom, Callahan and Hawkins (2011) conducted meta-analyses on the effect of APOE ε4 on cognition in healthy adults, both finding that ε4-carriers demonstrated poorer performance than non-carriers on measures of global cognitive function, executive function, and episodic memory. Additionally, Wisdom et al reported that perceptual speed was reduced for ε4-carriers. However, both these meta-analyses included predominately older-aged adults, and other age-stratified studies have failed to find an effect of APOE on cognitive function within any age group (Bunce et al, 2014; Jorm et al 2007). Rather than a detrimental effect, Rusted and colleagues (2013) reported superior cognitive function in young adult ε4-carriers. Tuminello and Han (2011) reviewed the literature from both healthy and dementia-related cohorts across the lifespan and found that young ε4-

carriers may have greater neural recruitment than non-carriers, but it was unclear whether this translated into improved cognitive function. There was also evidence of earlier and more rapid cognitive decline in older ε4-carriers, but preserved cognitive function in non-demented very-old (> 90 years) ε4-carriers, as compared to non-carriers. These contradictory findings have led some authors to suggest that any effect of APOE genotype on cognition may be domain-specific, and /or may be moderated by other environmental or biological factors (Jochemsen, Muller, van der Graff & Geerlings, 2012; Rusted et al., 2013; Small et al, 2004; Tuminello & Han, 2011, Wisdom et al., 2011).

Evidence for APOE ε4 impacting cognitive outcome following TBI is also mixed. While some reports have indicated that possession of APOE ε4 results in a detrimental effect on cognitive outcome following TBI (Anderson et al., 2009; Ariza et al., 2006; Crawford et al., 2002; Eramudugolla et al., 2014; Friedman et al., 1999; Müller et al., 2009; Noé, Ferri, Colomer, Moliner, & Chirivella, 2010; Sundström et al., 2004), others have failed to find any effects (Ashman et al., 2008; Chamelian, Reis, & Feinstein, 2004; Hodgkinson, Gillett, & Simpson, 2009; Isoniemi, Tenovuo, Portin, Himanen, & Kairisto, 2006; Liberman, Stewart, Wesnes, & Troncoso, 2002; Miller et al., 2010; Ponsford et al., 2011; Ponsford, Rudzki, Bailey, & Ng, 2007; Pruthi et al., 2010; Rapoport et al., 2008; Shadli, Pieter, Yaacob, & Rashid, 2011; Teasdale, Jorgensen, Ripa, Nielsen, & Christensen, 2000), and there have been reports that ε4-carriers may even display better cognitive outcomes following TBI than non-carriers (Han et al., 2007; Noé et al., 2010).

Unfortunately, there are a number of factors which make interpretation and comparison of the findings from the TBI literature problematic. Firstly, although TBI was recently defined as ‘an alteration on brain function, or other evidence from brain pathology, caused by an external force’ (Menon, Schwab, Wright & Maas, 2010, p.1637), it is recognised that alteration to function can be difficult to confirm when TBI is mild, or when

there has been a delay in the person presenting for medical assessment. Additionally, altered function may not become apparent until later in the recovery process and it can be unclear whether some symptoms have occurred as a direct result of a TBI, or are due to other events, such as orthopaedic injury or pharmacological treatment (Menon et al, 2010). These issues may lead to systematic differences between studies depending on the assessment protocols used at a given research/medical center, or as a result of differences in individual presentations. Furthermore, as there is no reliable biomarker of injury severity for TBI, it is necessary to estimate injury severity by using scales such as the Glasgow Coma Scale (GCS) or length of post-traumatic amnesia (PTA), which are based on behavioural responses post-injury, rather than underlying clinical pathology. While both are relatively reliable estimates of injury, there is a lack of correlation between severity estimates obtained from GCS as compared to PTA (Sherer, Struchen, Yablon, Wang, & Nick, 2008), and both have been found to have limited ability to predict functional outcomes both in early and later recovery (Ponsford, Draper, & Schonberger, 2008; Thornhill et al, 2000). Confounding factors such as poly-trauma, intoxication, existing premorbid conditions, medical interventions, or reliance on self-report can also impact negatively on injury estimation (Kemp, Agostinis, House, & Coughlan, 2010; Sherer et al., 2015; Zuercher, Ummenhofer, Baltussen, & Walder, 2009). A final issue in comparing studies is the substantial number of cognitive tests reported in the TBI literature, with a recent review revealing that a total of 263 cognitive tests had been employed in the adult TBI literature in the period between the years 2000 and 2012 alone (Tate, Godbee, & Sigmundsdottir, 2013). This array of tasks, in conjunction with the abovementioned injury and assessment-related issues, makes comparison both at an individual and group level, challenging.

As well as the issues relating to assessment of TBI severity and outcome, there have been growing concerns that the influence of genes on psychological function has been

exaggerated. Duncan and Keller (2011) found compelling evidence of a publication bias towards significant results in novel cGxE studies within psychiatric research, with further evidence that replication studies are more likely to be published either when positive results have been found, or when a novel finding is reported alongside a null finding. The authors also reported replication biases, insofar as samples sizes needed to be substantially larger for publication of non-significant replication studies. These findings indicate the effect of various genes may be being over-estimated. It has also been noted that many gene studies are underpowered, due to a combination of small samples sizes and/or small to modest effect sizes, and as a result may be prone to Type I errors (Dick et al., 2015; Duncan & Keller, 2011).

Cumming (2012) states that even when meta-analyses only include two studies, the margin of error is typically reduced by approximately 30%, and thus a meta-analytic approach may be particularly beneficial in cGxE studies to compensate for the issues described above (Dick et al., 2015). To date, two meta-analyses have investigated the effect of APOE ε4 on broad functional outcomes following TBI. One concluded that APOE ε4 may be associated with poorer outcomes in severe TBI, and that its detrimental effect may be pronounced in Asian populations (Zeng et al., 2014), with the second reporting that any detrimental effect of APOE ε4 may be more apparent in the post-acute recovery phase (Zhou et al., 2008). Both meta-analyses relied on broad or non-cognitive outcome measures, and were not intended to identify differences in cognitive function. Lawrence, Comper, Hutchison and Sharma (2015) recently conducted a systematic review of the effect of APOE ε4 across a range of outcomes, including neuropsychological function. The authors identified studies that included neuropsychological assessment, classifying each as providing evidence for APOE ε4 being hazardous, non-contributory, or protective. Of the 18 studies reviewed, the authors concluded that nine revealed no effect, seven reported a hazardous effect, and two

indicated a protective effect of APOE ε4 possession. Lawrence and colleagues also observed that memory impairment was found in all studies that identified APOE ε4 as being hazardous, and that APOE ε4 appeared to be more deleterious when TBI was classified as severe. Although memory was identified as a domain that may be more at risk of decline, it was not clear which other cognitive domains were assessed, or the magnitude of effect for any of the neuropsychological functions that were assessed.

The aim of our study is to explore the effect of APOE ε4 on cognitive recovery in the first 12 months following TBI, in adult populations. Using a meta-analytic approach will enable us to explore whether there is any effect of APOE ε4 on cognitive impairment following TBI, beyond the memory deficits identified in Lawrence et al.'s review, and to estimate the magnitude of effect within both general cognitive function and specific cognitive domains. Given the aforementioned issues regarding the range of tasks employed in this area, we attempted to attain a balanced approach by undertaking a series of meta-analyses. First, we explored the effect of APOE ε4 in general cognitive function by including all studies which have employed neuropsychologically validated tests. Second, to address the issue of heterogeneity, we conducted domain-specific meta-analyses in which we selected tasks that have been recommended for cognitive assessment following TBI (Bagiella et al, 2010; Lezak, Howison & Loring, 2004; Wilde et al., 2010). The domains of interest were executive function, working memory, visual memory and verbal memory, as these are known to be the most commonly impaired in TBI (Mathias & Wheaton, 2007; Rabinowitz & Levin, 2014; Rohling et al., 2011).

Method

Literature search and selection

The search and selection of articles was conducted according to the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA; Moher, Liberati, Tetzlaff, & Altman, 2009). Pubmed (MEDLINE), Web of Science, and Proquest (inc PsychINFO) were searched for articles published between 1980 and 29th April 2015. The search terms used were 'apolipoprotein e' 'APOE' 'brain injury' 'TBI' 'head injury' 'cognitive' 'memory' 'executive function' and 'neuropsychological', with both long and short versions included (a wildcard term was added pre and post word stem for short versions). A total of 445 publications were obtained, and were screened for duplications (*n* = 134), resulting in 311 studies.

Inclusion criteria were as follows; the study must be an original report in full text, containing adult TBI participants categorised by APOE genotype (ε4-carriers versus non-carriers), with neuropsychological assessments undertaken within 12 months of injury. Publications reporting any level of TBI severity (mild, moderate, and/or severe) were included. A total of 258 studies were excluded as they did not use adult TBI participants, or participants had not been APOE genotyped, 24 were found to be either non-original studies, or were not full-text. Another 11 studies either did not use cognitive-specific outcome measures, or did not measure the domains of interest within 12 months of injury, and one study was excluded as it used a duplicate population that was already included in the meta-analysis. This resulted in 17 publications meeting the inclusion criteria. Seven studies did not have sufficient data published to calculate effect size, and attempts were made to contact corresponding authors. We were unable to obtain data for these studies and so ten studies were included in the meta-analysis. A flowchart for the search strategy and study selection is available in the supplemental information section which follows the chapter discussion. We

also contacted authors when the included studies did not stratify demographic and injury data by APOE ε4 status.

Where a longitudinal design had been employed, only baseline measurements were included. One study (Noé et al., 2010) reported separately on the cognitive function of participants who were still in PTA, and those who had emerged from PTA. Only the findings for those participants who had emerged from PTA are reported here.

Test selection

General cognitive function.

All ten studies were included in the general cognitive function analysis. Validated tests of general ability, psychomotor function, attention, executive function, working memory, verbal memory, and visual memory were included, resulting in a total of 33 tasks across the seven domains, as shown in Table 3.

Table 3

Cognitive domains, tests, and associated studies included in the general cognitive functioning meta-analysis

Domain	Test[a]	Studies
General Ability	FIM (Cognition)	Hodgkinson et al; Lichtman et al.
	MR	Han et al; Hodgkinson et al; Shadli et al.
	NRS-R (Executive/cognition)	Ariza et al.
	WAIS PIQ	Anderson et al.
	WAIS VIQ	Anderson et al.
Psychomotor Speed	CALCAP	Hodgkinson et al.
	CRT	Chamelian et al; Liberman et al.
	Digit Symbol	Han et al.
	Grooved Pegboard	Ariza et al; Liberman et al.
	SDMT	Liberman et al.
	SRT	Chamelian et al; Liberman et al.
	Tapping	Anderson et al.
Attention	PASAT	Chamelian et al; Han et al; Liberman et al.
	Divided Attention	Liberman et al.
	Number Vigilance	Liberman et al.
Executive Function	Fluency - Animal	Crawford et al.
	- Design	Han et al.
	- Word	Ariza et al; Chamelian et al; Crawford et al; Han et al; Shadli et al.
	SCWT	Han et al; Liberman et al.
	Sorting Test	Han et al.
	TMT B	Ariza et al; Han et al; Shadli et al.
	WCST	Chamelian et al; Shadli et al.

Continued overleaf...

Domain	Test[a]	Studies
Working Memory	DS	Han et al; Hodgkinson et al.
	DSB	Hodgkinson et al.
	WMI	Chamelian et al; Noé et al.
Verbal Memory	CVLT (immediate and delayed)	Crawford et al (immediate only); Chamelian et al; Han et al.; Noé et al.
	CVLT Trial 5 total	
	RAVLT (immediate and delayed)	Crawford et al.
	SRCL	Ariza et al; Hodgkinson et al; Shadli et al.
	WMS (logical memory, immediate and delayed)	Anderson et al.
		Chamelian et al; Han et al.
	Word recall	Liberman et al.
Visual Memory	CFT (immediate and delayed)	Ariza et al (immediate only); Hodgkinson et al.
	BVMT (immediate and delayed)	Chamelian et al.

[a]BVMT, Brief Visuospatial Memory Test; CALCAP California Computerised Assessment Package; CFT, Complex Figure Test; CRT, Choice Reaction Time; CVLT, California Verbal Learning Test; DS, Digit Span; DS-B, Digit Span Backwards; FIM, Functional Independence Measure; MR, Matrix Reasoning; NRS-R, Neurobehavioral Rating Scale – Revised; PASAT, Paced Auditory Serial Addition Test; RAVLT, Rey's Auditory Verbal Learning Test; SCWT, Stroop Colour-Word Test; SDMT, Symbol Digit Modalities Test; SRCL, Selective Reminding Test; SRT, Simple Reaction Time; TMT B, Trail-Making Test B; WAIS PIQ, Wechsler Adult Intelligence Scale – Performance Intelligence Quotient; WAIS VIQ, Wechsler Adult Intelligence Scale – Verbal Intelligence Quotient; WCST, Wisconsin Card Sorting Test; WMS, Wechsler Memory Scale

In order to reduce heterogeneity, only tasks recommended for assessment of outcome in TBI were selected for the specific cognitive domains of interest (Bagiella et al, 2010; Lezak, Howison & Loring, 2004; Wilde et al., 2010). These domains were chosen a priori as they are reported to be most sensitive to change following TBI, regardless of severity of injury (Mathias & Wheaton, 2007; Rabinowitz & Levin, 2014; Rohling et al., 2011). The studies and tasks included for each cognitive domain are described below:

Executive function.

Five studies were included in the executive function meta-analysis (Ariza et al, 2006; Chamelian et al, 2004; Crawford et al, 2002; Han et al, 2007, Shadli et al, 2011). Three tests were employed; the Trail Making Test B (TMTB), the Controlled Oral Word Association Task (COWAT; verbal fluency only), and Wisconsin Card Sorting Test (WCST). For the WCST two measures (total and number of perseverative errors) were obtained, resulting in a total of four measures being included in this domain. As higher scores on the TMTB and WCST perseverative errors indicate poorer performance, the direction of effect size was reversed on these tasks.

Working memory.

Four studies were included in the working memory meta-analysis (Chamelian et al, 2004; Han et al, 2007; Hodgkinson et al, 2009; Noé et al, 2010). Digit Span (DS) score was obtained from Han et al., and Hodgkinson et al., with Hodgkinson et al also providing a Digit Span Backwards (DSB) score. Chamelian et al. and Noé et al. provided a composite working memory index score (WMI) based on Digit Span, Letter-Number Sequencing, and Arithmetic scores from the Wechsler Adult Intelligence Scale.

Verbal memory.

Seven studies were included in the verbal memory meta-analysis (Ariza et al, 2006; Chamelian et al, 2004; Crawford et al, 2002; Han et al, 2007, Hodgkinson et al, 2009; Noé et al, 2010; Shadli et al, 2011). Three tests were used; the Californian Verbal Learning Test (CVLT), the Rey Auditory Verbal Learning Test (RAVLT), and the Wechsler Memory Scale Logical Memory Task (WMS). Immediate and delayed recall scores were obtained for each task, resulting in a total of six measures.

Visual memory.

Three studies were included in the visual memory meta-analysis (Ariza et al, 2006; Chamelian et al, 2004; Hodgkinson et al, 2009). Two tasks were included; the Complex Figure Test (CFT) and the Brief Visuo-Spatial Memory Test (BVMT). Immediate and delayed recall scores were obtained for each task, resulting in four measures.

Statistical analysis

Between group means and standard deviations were used to calculate Cohen's *d*. Ariza et al., (2006) and Han et al., (2007) did not have raw descriptive data for some tasks, and so Cohen's *d* was calculated using the *F* or *t* statistic and sample size in these instances (for formulae see Lipsey & Wilson, 2001). Meta-analysis was conducted using the Exploratory Software for Confidence Intervals programme (ESCI; Cumming, 2012). Effect size estimates for each domain in the general cognition meta-analysis, and for each task in the domain-specific meta-analyses were pooled, and were also combined to determine the overall effects. Due to the relatively small sample size, a fixed effects model was used. It is important to note that random effects models are generally recommended where heterogeneity is expected (Cumming, 2012). However, random effects models are also

associated with reduced power (Howell, 2013), and given the small number of studies included in our meta-analysis it was decided that a fixed effects model, reporting the heterogeneity, was the most pragmatic approach. The Q statistic and associated I^2 value were calculated to test the assumption of homogeneity of effect sizes (Howell, 2013). A significant Q value indicates heterogeneous effect sizes, and I^2 estimates the percentage of variation across the studies occurring as a result of heterogeneity.

Results

Demographic and injury-related characteristics

The ten studies that were included in the analysis resulted in a combined sample of 143 ε4-carriers and 510 non-carriers. While genotype frequencies were reported for most studies, all studies analysed data by classifying participants as either APOE ε4 carriers or non-carriers. Demographic and injury-related characteristics for each study are provided in Table 4. As can be seen, we were unable to ascertain the average age of APOE ε4 and non-carriers for one study (Liberman et al., 2002), sex ratio for two studies (Crawford et al., 2002; Shadli et al., 2011), or ethnicity for four studies (Anderson et al, 2009; Ariza et al., 2006; Hodgkinson et al., 2009; Shadli et al., 2011), as stratified by APOE ε4 status.

Table 4

Demographic and injury-related characteristics of studies included in the meta-analysis

Author	Country	n		Injury severity	Mean time since injury	Mean age (SD)		% Female		% Caucasian	
		ε4-carriers	non-carriers			ε4-carriers	non-carriers	ε4-carriers	non-carriers	ε4-carriers	non-carriers
Anderson et al. (2009)	USA	15	36	Mild to severe	1 month	38.2 (10.1)	33.9 (11.8)	13.00	11.00	NR	NR
Ariza et al. (2006)	Spain	10	67	Moderate to severe	215 (23) days	34.70 (18.31)	28.87 (11.47)	30.00	20.90	NR	NR
Chamelian et al. (2004)	Canada	19	71	Mild	6 months	31.20 (13.3)	34.10 (12.3)	47.40	38.00	73.70	77.50
Crawford et al. (2002)	USA	30	80	Mild to severe	41 (33) days	32.25 (11.57)	33.56 (14.18)	NR	NR	53.33	35.00
Han et al. (2007)	USA	16	62	Mild to moderate	38 (13) days	22.56 (3.76)	25.26 (5.78)	18.70	3.23	75	79.50
Hodgkinson et al. (2009)	Australia	13	45	Moderate to severe	12 months	40.33 (14.12)	31.08 (13.96)	38.5	22.2	NR	NR
Liberman et al. (2002)	USA	18	62	Mild to moderate	3 weeks	NR	NR	27.80	43.50	83.30	80.60
Lichtman et al. (2000)	USA	7	24	Moderate to severe	6 months	39.8 (18.1)	34.3 (18.1)	28.57	29.16	85.14	87.50
Noé et al. (2010)	Spain	9	50	Moderate to severe	312 (431) days	28.45 (8.96)	29.70 (10.92)	28.57	42.86	100	100
Shadli et al. (2011)	Malaysia	6	13	Mild to moderate	6 weeks	25.00 (8.63)	26.15 (6.84)	NR	NR	NR	NR

NR = Not reported.

One study compared the TBI groups to healthy controls, matched on age, education, and general ability (Shadli et al., 2011); the remaining nine studies included only TBI sufferers. All studies compared age of ε4-carriers and non-carriers, with only Ariza et al. reporting significant age differences, which were controlled for by ANCOVA. With the exception of Crawford et al. (2002) and Shadli et al. (2011), all studies reported that they compared sex ratios for ε4 carriers and non-carriers, with no significant differences found. Education level was also compared for each group, other than Hodgkinson et al. (2009), with no significant differences being reported. Six studies recruited from North American populations (Anderson et al., 2009; Chamelian et al., 2004; Crawford et al., 2002; Han et al., 2007; Liberman et al, 2002; Lichtman et al, 2000), two studies were undertaken in a Mediterranean population (Ariza et al., 2006; Noé et al., 2010), one study was undertaken in an Asian population (Shadli et al., 2011), and one in an Australian population (Hodgkinson et al., 2009).

Injury severity was estimated by GCS and/or PTA in most studies, however Anderson and colleagues (2009) used time to follow commands. There was also variation in how injury severity was classified. Ariza et al. (2006), Chamelian et al. (2004) and Han et al. (2007) used GCS score to categorise injury severity as mild (13-15) moderate, (9-12) or severe (<8), with Chamelian et al. and Han et al. also incorporating PTA into their estimates of severity (<24 hrs = mild, 1-7 days = moderate, > 7 days = severe). Liberman et al (2002) stratified GCS scores into three levels (9-12, 13-14, and 15) but did not identify these in terms of severity. Noé et al. (2010) also categorised their participants as having sustained a moderate or severe TBI based on GCS, however, while mean GCS scores are provided, it is not clear what cut-offs were employed. Similarly Shadli et al. (2011) report a mild-moderate cohort based on mean a GCS of 13.21 (*SD* = 1.99). Crawford et al. (2002) stratified each group based on length of PTA (< 1 hr, 1-24 hrs, 1-7 days, 7-30 days, and > 30 days), but did not

explicitly categorise severity. Hodgkinson et al. (2009) estimated injury severity based on PTA using the Modified Oxford PTA scale (1-7 days = severe, 7-28 days = very severe, > 28 days = extremely severe). As all of the participants in Hodgkinson et al's study had PTA >1 day, they were classified as having severe TBI, however, as others have consistently classified 1-7 days as moderate, the injury severity of this cohort has been identified as moderate to severe in Table 1. There were no significant differences in injury severity between APOE ε4 carriers and non-carriers reported in any of the studies with the exception of Lichtman et al (2000), who reported that non-carriers had significantly longer coma period than ε4-carriers. This was adjusted for in the subsequent analysis.

Meta-analysis results.

Ten studies were included in the general cognitive function meta-analysis (ε4-carriers n = 143, non-carriers n = 510). There was an overall effect of d = 0.038 (95% CI [-0.028, 0.105]) indicating that there were no differences in overall cognitive function. Although there was substantial variability between the domains, heterogeneity was not significant ($Q(6) = 6.686$, $p = .351$; $I^2 = 10.26\%$). Figure 2 depicts the forest plots for general cognitive function.

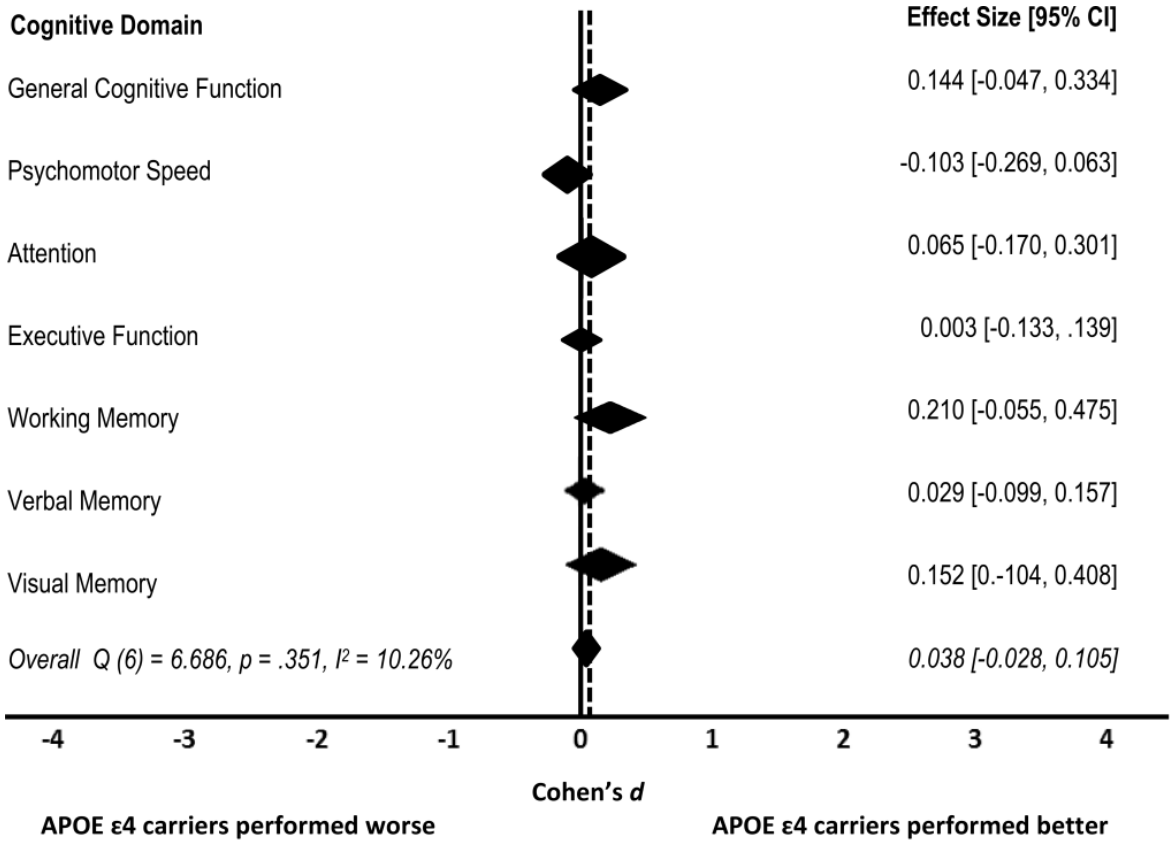


Figure 2. Forest plot for general cognitive outcome Cohen's d effect sizes and 95% CIs. Dotted vertical line indicates overall pooled effect size.

The executive function tasks of interest in the domain specific studies (TMTB, COWAT, and WCST) were employed in five studies (ε4-carriers *n* = 81, non-carriers *n* = 293). There was an overall effect of *d* = -0.118 (95% CI [-0.305, 0.069]), suggesting no difference between the performance of ε4-carriers and non-carriers. There was a significant effect for heterogeneity (Q (10) = 33.038, *p* = .001; I^2 = 69.7%). The only task found to have significant heterogeneity was the COWAT, and so this was removed and the data was re-analysed, resulting in an increase in overall effect; *d* = -0.145 (95% CI [-0.394, 0.105]). However, heterogeneity remained significant (Q (6) = 21.738, *p* = .001; I^2 = 72.4%). The forest plots for executive function tasks are depicted in Figure 3.

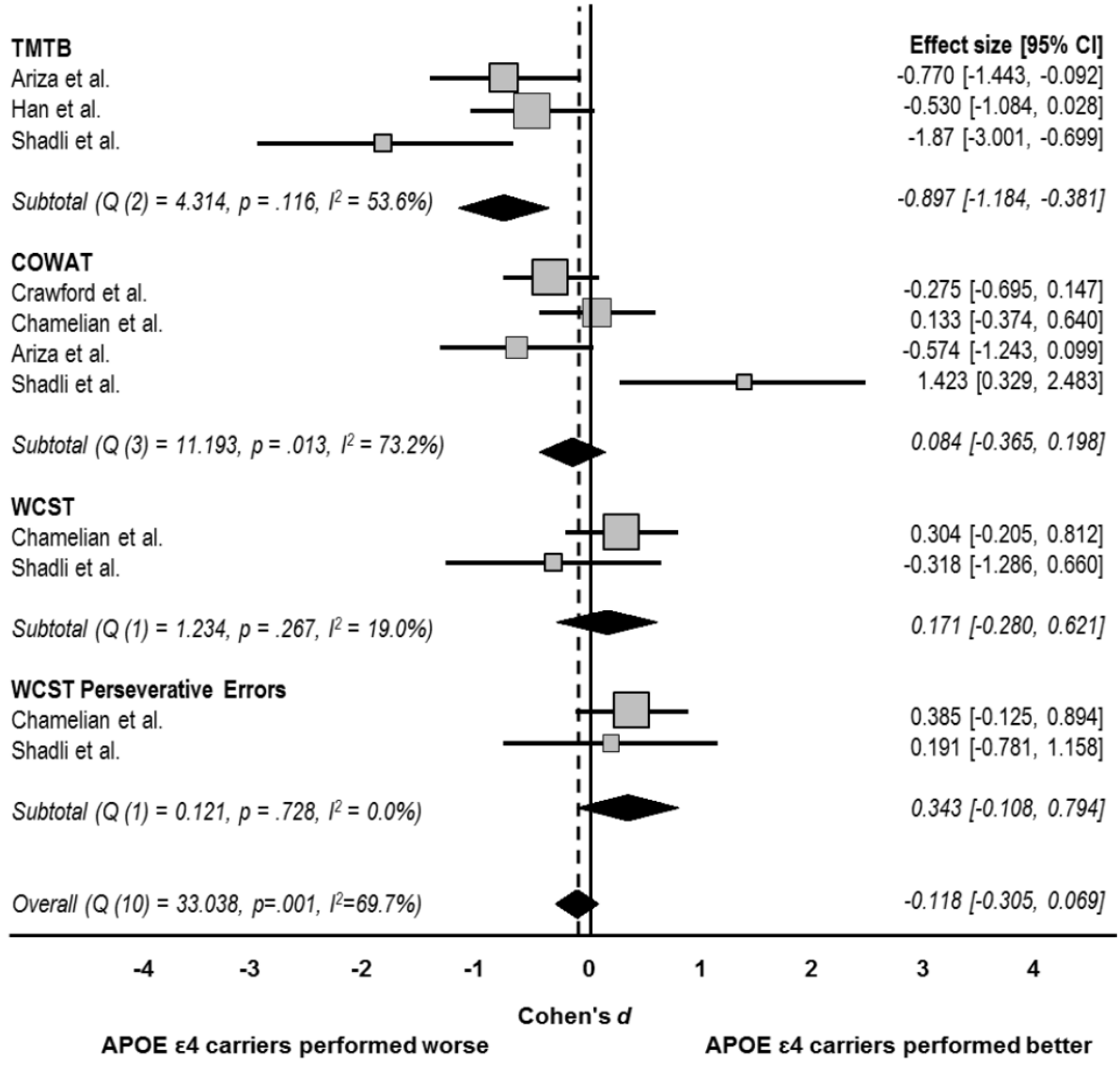


Figure 3. Forest plot for executive function Cohen's d effect sizes and 95% CIs. Dotted vertical line indicates overall pooled effect size.

Four studies assessed working memory (ε4-carriers n = 57, non-carriers n = 228), using the DS and/or DSB, or the WMI. A small overall effect of d = 0.210 (95% CI [-0.055, 0.476]) was found, indicating no differences between ε4-carriers and non-carriers. There was also a significant effect for heterogeneity (Q (4) = 10.600, p = .031; I^2 = 62.3%). Figure 4 displays the forest plots for the working memory tasks.

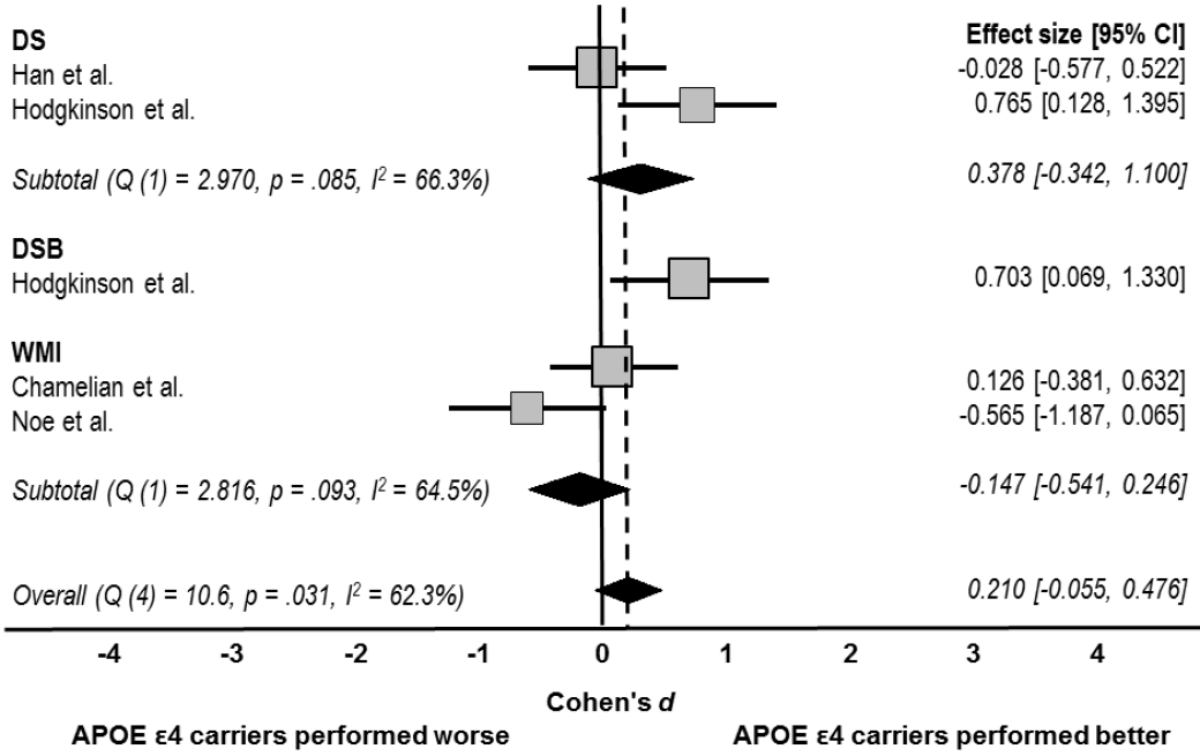


Figure 4. Forest plot for working memory Cohen's d effect sizes and 95% CIs. Dotted vertical line indicates overall pooled effect size.

Verbal memory was the domain most frequently assessed, with seven studies including a verbal memory measure of relevance to the domain-specific meta-analysis (ε4-carriers n = 90, non-carriers n = 343). These being the CVLT (immediate and delayed recall), the RAVLT (immediate and delayed recall), or the WMS logical memory tasks (immediate and delayed recall). There was a small overall effect of d = 0.061 (95% CI [-0.081, 0.204]), indicating negligible differences between ε4-carriers and non-carriers. There was also a significant effect for heterogeneity (Q (16) = 49.719, p = .001; I^2 = 67.8%). Figure 5 demonstrates the forest plots for verbal memory tasks.

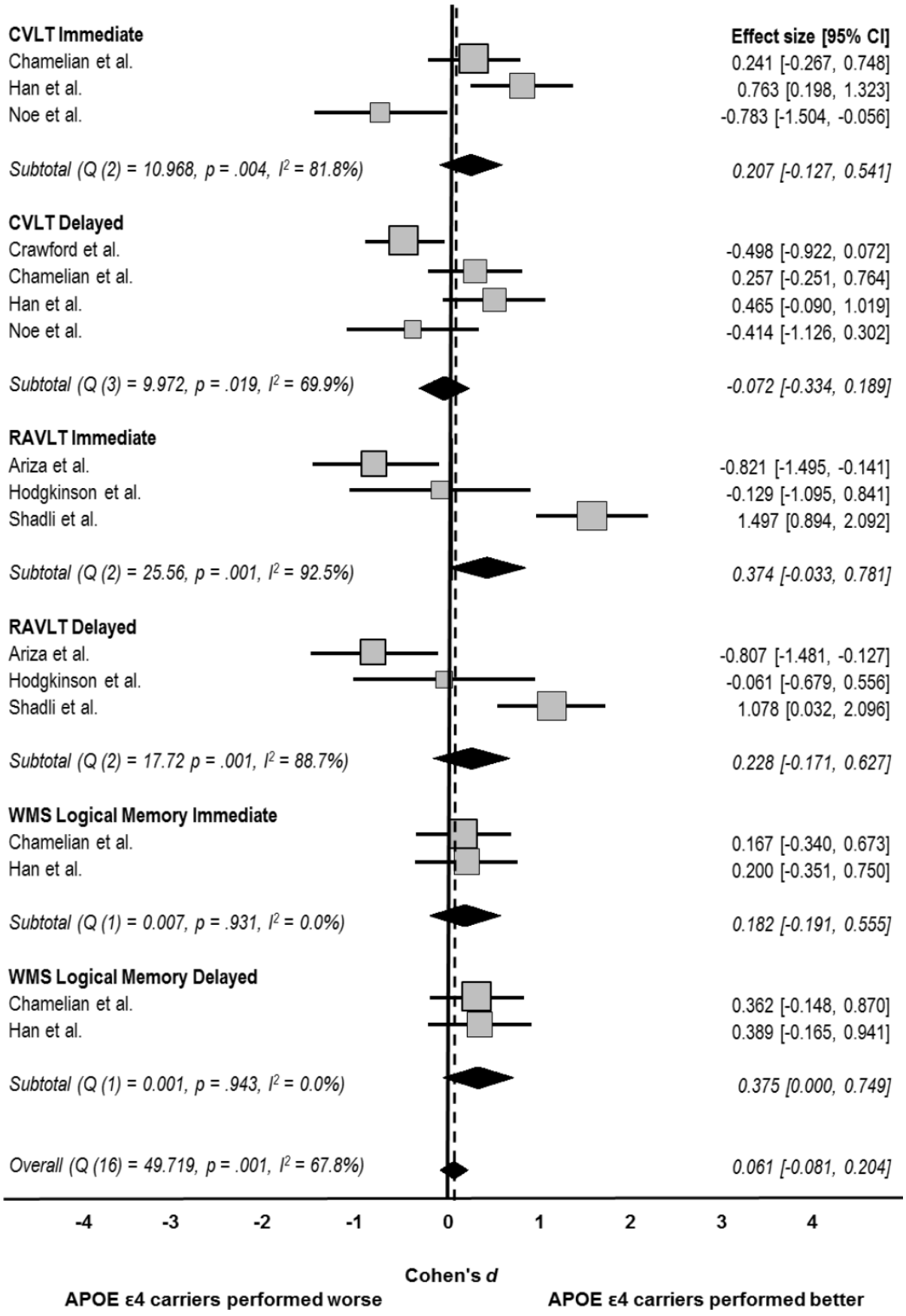


Figure 5. Forest plot for verbal memory Cohen's d effect sizes and 95% CIs. Dotted vertical line indicates overall pooled effect size.

Three studies used tasks assessing visual memory (ε4-carriers *n* = 42 non-carriers *n* = 183), using either the CFT (immediate and delayed recall) or BVMT (immediate and delayed recall). There was an overall effect size of *d* = 0.152 (95% CI [-0.104, 0.409]), suggesting no significant differences between ε4-carriers and non-carriers. There was a non-significant effect for heterogeneity (Q (4) = 5.200, *p* = .267; I^2 = 23.1). The forest plots for visual memory are shown in Figure 6.

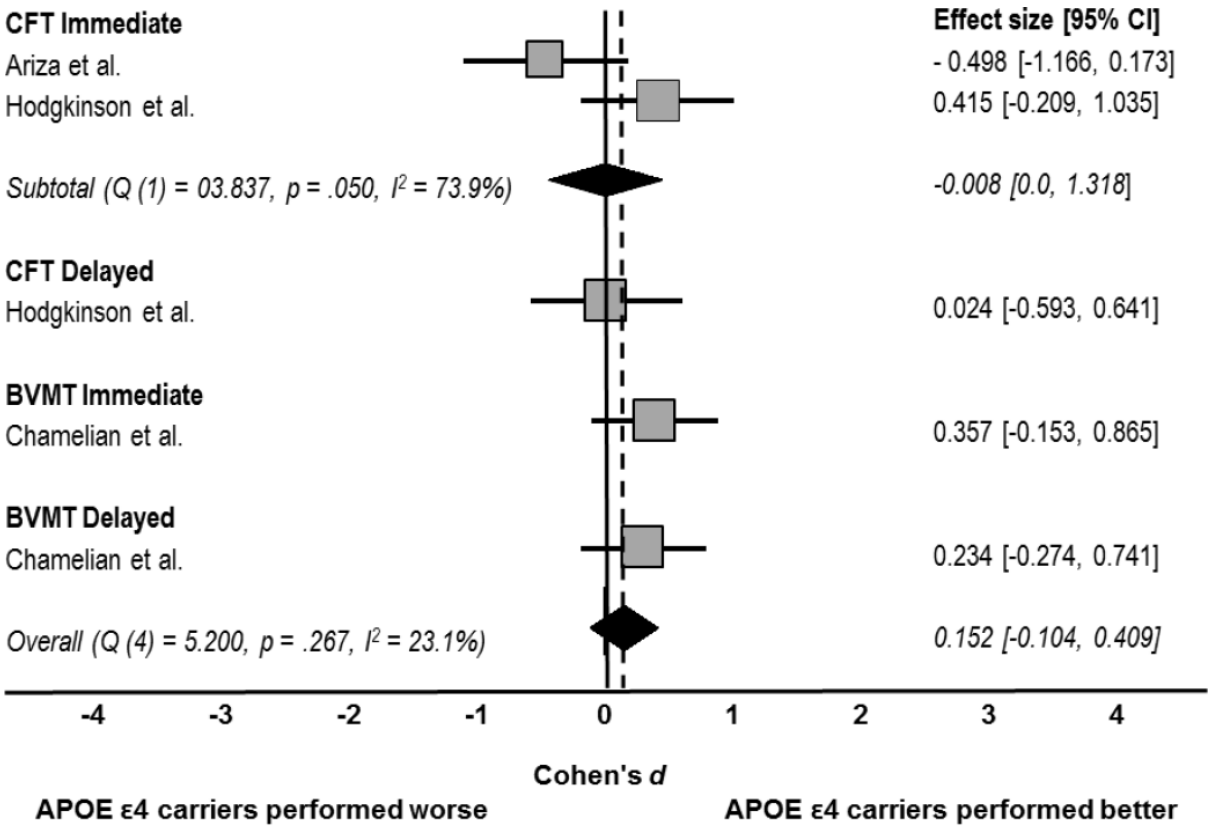


Figure 6. Forest plot for visual memory Cohen's d effect sizes and 95% CIs. Dotted vertical line indicates overall pooled effect size.

Given the evidence of variation of ε4 frequency and expression in different ethnic groups (Corbo & Scacchi, 1999; Farrer et al., 1997; Zeng et al., 2014), additional analysis was conducted in which Shadli et al's (2011) study was excluded as the study recruited from an Asian population. As Shadli et al did not use measures of working memory or visual

memory, re-analysis was only conducted on the executive function and verbal memory domains. This resulted in a non-significant increase in effect size for executive function (*d* = -0.122 [-0.323, 0.078]), and a slight reduction in effect size for verbal memory (*d* = 0.069, 95% CI [-0.082, 0.221]), suggesting there was minimal effect due to ethnicity.

Discussion

This is the first meta-analysis to be conducted on the effect of APOE ε4 on cognitive function following TBI. Meta-analytic approaches are known to reduce margin of error (Cumming, 2014), and therefore it is hoped that the current findings provide a more accurate estimation of the effect of APOE ε4 on post-TBI cognitive function. The findings from the general cognitive function meta-analysis and the domain-specific meta-analyses revealed similar results. There were no significant differences between APOE ε4-carriers and non-carriers in general cognitive function, and the domain-specific meta-analyses revealed no significant differences between ε4-carriers and non-carriers for any of the domains, despite the selection of tasks known to be sensitive to change following TBI. Our findings do not appear to align with the recent review by Lawrence et al (2015), in which possession of APOE ε4 was found to be associated with poorer memory function following TBI. However it must be noted that half the studies described by Lawrence and colleagues found a non-contributory effect of APOEε4, thus is it is possible that using a meta-analytic approach was able to provide a more integrative interpretation of the literature to date. Lawrence et al also included studies of late outcome, whereas we focussed on outcomes in the first 12 months post injury. Therefore another possible explanation for the difference between our and Lawrence et al's findings is that any detrimental effect of APOE ε4 may be more apparent in later recovery.

A number of factors must be considered in interpreting this finding. Firstly, the small effect sizes and relatively small sample sizes that our meta-analyses are based upon means that the current study may be underpowered, and it must also be acknowledged that when there is publication bias in a given topic, meta-analysis will reflect the inflated positive findings (Duncan & Keller, 2011). Thus, while this analysis offers a more considered interpretation of the findings to date, the results reported here must be viewed cautiously.

Our meta-analysis also included studies in which injuries ranged from mild to severe, but there is evidence from both animal and human studies indicating that APOE ε4 may have a greater impact when TBI is severe (Mannix et al., 2013; Millar et al., 2003), and a recent meta-analysis comparing general prognosis for APOE ε4 carriers and non-carriers also noted that APOE ε4 carriers only appeared to be disadvantaged when injury was severe (Zeng et al., 2014). This was also observed by Lawrence and colleagues (2015) in their recent review. Interestingly, Maxwell and colleagues (2011) report that ε4-carriers may be at greater risk of experiencing brain micro-bleeds, and there is evidence that the presence of micro-bleeds in TBI sufferers is a strong predictor of cognitive impairment (Fagerholm, Hellyer, Scott, Leech, & Sharp, 2015). There is also emerging evidence that APOE ε4 is associated with greater neuro-inflammation (Cudaback, Yang, Montine, & Keene, 2015; Tai et al., 2015), although this is yet to be demonstrated in TBI populations. Whether such allele-specific effects translate to more severe initial pathology in TBI, and/or a protracted recovery period, remains unknown. Should the effects of APOE ε4 be more pronounced when injury is severe, or alternatively, lead to more severe pathology, it is possible that these effects would not be uncovered when mild to moderate TBI cohorts are included in analyses.

Demographic data obtained from the included studies also indicates that participants were primarily young to middle-aged adults. It has long been established that APOE ε4 is associated with age-related neurological disorders such as Alzheimer's disease (Corder et al.,

1993), indicating that any detrimental effect of the ε4 allele may be only be apparent in older adults. Thus, the apparent contradiction between the findings in this meta-analysis and the evidence for a detrimental effect of APOE ε4 might be due to age-associated effects, which we were unable to assess. A potential explanation for the apparent age-associated variation is that the APOE gene operates via an antagonistic pleiotropic mechanism, whereby it exerts beneficial effects upon a given characteristic before and during the reproductive phase of life, but is deleterious in post-reproductive life (Carter & Nguyen, 2011). This mechanism is yet to be clearly demonstrated for the APOE gene, however some authors have suggested this explains the relationship between APOE ε4 and Alzheimer's disease (Han & Bondi, 2008; Leroi et al., 2005; Tuminello & Han, 2011), and there is some evidence that APOE ε4 has an antagonistic pleiotropic effect on a range of non-cognitive traits (Kulminski et al., 2013; Kulminski et al., 2011; Wierenga et al., 2013), and possibly on cognitive function in some clinical cohorts (Chang et al., 2011).

A further issue for our meta-analysis is the differences between studies in method of assessing injury severity. The majority of studies included here provided GCS scores, however two studies relied on PTA to classify injury severity (Crawford et al., 2002; Hodgkinson et al., 2009), and a third used time to follow commands as an estimate of severity (Anderson et al., 2009). While both GCS and PTA have been shown to be adequate estimates of injury severity, severity scores obtained from the GCS have been found to be poorly correlated with severity estimated by PTA (Sherer et al., 2008), and there is often variation in the timing of GCS administration, which can lead to over- or under-estimation of injury severity (Zuercher et al., 2009). As a result there may be substantial variation in the level of injury severity both between and within individual studies, and caution must be applied when comparing injury severity classifications between studies.

A previous meta-analysis by Zeng and co-workers (2014), using non-cognitive outcome measures, also indicated that ethnicity can influence both frequency and expression of the APOE ε4 allele, with some evidence that APOE ε4 is associated with greater risk of neurological dysfunction in some Asian populations. When we removed the only study in our meta-analysis that had recruited from an Asian population (Shadli et al., 2011), there was no evidence of systematic changes. Although this suggests that ethnicity was unlikely to have influenced this meta-analysis, given the small sample size, this finding should not be applied to the broader TBI population.

Directions for future research.

There are a number of issues that need to be addressed in future research, and a key theme is that reporting demographic, injury and outcome-related data, stratified by APOE genotype (or at least by APOE ε4 status) will allow better understanding of the role of APOE in cognitive recovery following TBI. Inclusion of this information (either in publications or attached as supplementary information) will be of benefit to meta-analytic or other integrative approaches.

As stated previously, the wide range of tasks employed to assess cognitive function after TBI has made comparison of findings challenging. Ideally, as well as using neuropsychological tasks that are psychometrically validated, repeatable, and sensitive to change following TBI, routine incorporation of a set of core neuropsychological tests would mean that individual studies can be included into meta-analytic or other approaches, permitting more rigorous exploration of the cognitive domains known to be impacted by TBI. Wilde and colleagues (2010) propose a three tier approach to functional assessment following TBI, with specific recommendations for neuropsychological assessment. In the Wilde et al protocol, the first tier contains a number of core tasks, allowing a time-efficient but broad

coverage of cognitive domains known to be impacted by TBI; the second supplemental tier provides additional tasks to allow a more in-depth exploration when specific domains are of interest; and the third tier includes emerging, less well-established tests, thus allowing newer tests to become validated. While it is beyond the scope of the current study to describe Wilde et al's protocol in detail, it is suggested that future researchers routinely incorporate the core tasks recommended by Wilde et al, and preferably the supplemental and emerging tasks, alongside any other measures of interest.

We also recommend the inclusion of both GCS and PTA when possible. While the GCS is the most frequently used scale, recent evidence indicates that PTA may be a more accurate estimation of injury severity, and a better predictor of functional outcome (Perrin et al., 2015; Schonberger, et al., 2009), although caution must be applied where PTA has been self-reported (Kemp et al., 2010; Sherer et al., 2015). Given that GCS is routinely administered in the emergency medicine setting, it is likely that this will remain the most commonly used measure for some time, however inclusion of both GCS and PTA will permit investigation of the relationship between GCS and PTA, and will improve the ability to interpret and compare injury severity between studies, and clarify the relationship between each scale and functional outcome.

Given the evidence that possession of APOE ε4 may be associated with reduced cognitive function in the general population (Tuminello & Han, 2011; Wisdom, Callahan, & Hawkins, 2011), further research is needed to establish whether any differences in cognitive impairment between ε4-carriers and non-carriers are a result of interaction between TBI and APOE genotype, or are a reflection of premorbid differences in cognitive function. To date, only one study has compared pre and post-TBI cognitive function of ε4-carriers (Sundström et al., 2004), and another (included in our analysis) has compared healthy ε4-carriers to ε4-carriers who had sustained a TBI (Shadli et al., 2011). In the pre- and post-TBI comparison

by Sundström and colleagues, it was found that ε4-carriers displayed greater post-injury cognitive declines than non-carriers, as compared to their pre-injury test performance, suggesting that APOE ε4 confers a greater vulnerability to cognitive impairment arising from TBI (Sundström et al., 2004). Alternatively, Shadli et al. (2011) compared the cognitive function of healthy controls to a mild-moderate TBI sample, with both groups categorised as either ε4-carriers or non-carriers. Shadli and colleagues reported no significant differences across a range of cognitive tasks, with the exception of poorer performance of the ε4-TBI group as compared to the ε4-healthy control group on the TMTB. However, as stated above, it is possible that any detrimental effects of APOE ε4 may be more pronounced in severe TBI, and it is therefore recommended that further exploration of this issue is needed to clarify the interaction between APOE genotype and TBI.

It is also recommended that a more nuanced approach to exploring the APOE gene is needed. In particular, we suggest that moderating factors need to be further explored. As mentioned in the previous section, injury severity may influence the effect of APOE ε4 on cognitive function after injury (Lawrence et al., 2015; Mannix et al., 2013; Millar et al., 2003), and this warrants further exploration. Furthermore, if APOE ε4 is expressed via an antagonistic pleiotropic mechanism, it is possible that TBI would have more severe effects for APOE ε4 carriers if sustained in later life, but this hypothesis is yet to be tested in the TBI population. There is tentative support for this mechanism from animal studies, where it has been reported that APOE ε4 is associated with poorer cognitive performance in older injured animals, but not in juveniles (Mannix et al., 2011). Others have reported that APOE ε4 may be associated with improved cognitive performance in paediatric TBI cohorts (Blackman et al., 2005; Moran et al., 2009). However, there have only been two studies which specifically explored the interaction between age and APOE genotype in adult TBI cohorts. One reported no age-related interactions (Friedman et al., 1999), and the other which revealed decreased

differences between ε4-carriers and non-carriers cognitive function in later life (Eramudugolla et al., 2014). Additionally, Rapoport and colleagues (2008) reported no significant differences in cognitive function in ε4-carriers and non-carriers who had sustained a TBI in later life. There is also some evidence from animal and in-vitro studies which indicates the expression of APOE is moderated by sex hormones (Koutseff, Mittelhaeuser, Essabri, Auwerx, & Meziane, 2014; Raber et al., 1998; Struble, Nathan, Cady, Cheng, & McAsey, 2007). To date, only two studies have directly investigated the potential interaction between sex and APOE, both of which used broad outcomes measures and therefore were not included in the current meta-analysis. Ponsford et al. (2011) reported that in their TBI sample, female ε4-carriers over the age of 55 had poorer outcomes than males of the same age, or than female non-carriers (as measured by the Extended Glasgow Outcome Scale). In contradiction, Alexander and colleagues (2007a) report no interaction between sex and APOE status using the same outcome measure. While we acknowledge that individual studies may lack the power to detect such interactions, routine inclusion of basic demographic and injury related data, stratified by genotype (or at least by presence/absence of APOE ε4), will allow exploration by meta-analysis or other integrative approaches once a sufficient number of studies are published. These factors should be included routinely, regardless of whether there is any a priori evidence, as the effect of a given characteristic may not initially be apparent.

There has also been minimal exploration of APOE ε4 dose-dependent effects on cognition in the TBI population. In the only study comparing ε4-homozygotes to ε4-heterozygotes to date, Ponsford and colleagues (2007) observed that ε4 homozygotes appeared to have greater initial cognitive impairment following TBI, although interpretation was hampered by a small sample size. As the majority of ε4-carriers are heterozygous there may be limits to determining the effect of the ε4 allele in isolation. Nevertheless, comparison

of ε4-homozygotes to other groups may assist in determining whether the ε4 allele is indeed detrimental to cognitive recovery following TBI.

The majority of studies also dichotomise participants as either ε4-carriers (possessing a genotype of ε2/ε4, ε3/ε4 or ε4/ε4) or non-carriers (ε2/ε2, ε2/ε3, or ε3/ε3). However, there is emerging evidence that the ε2 allele is dominant over the ε3 and ε4 alleles, and may confer protection following insult or injury (Corder et al., 1994; Laskowitz, Horsburgh, & Roses, 1998; Mahley & Rall 2000; Miller et al., 2010). Thus, inclusion of individuals with an ε2/ε4 genotype may mask any deleterious effects of ε4, and alternately the inclusion of ε2 carriers in the non-ε4 carrier cohort may attenuate differences between ε4-carriers and non-carriers. It could be argued that given the rarity of the ε2 allele it is unlikely to have a meaningful impact, but given the small sample sizes often used, and the modest effect sizes that have been reported here, it may be appropriate to exclude such individuals, as indeed has been done in some more recent studies (Eramudugolla et al., 2014; Isoniemi et al., 2006; Ponsford et al., 2007). It has also been suggested that the potential effect of APOE ε2 should be considered separately, given the potential protective properties of this allele (Suri, Heise, Trachtenberg, & Mackay, 2013). The relative scarcity of the ε2 allele may make analysis of results from an individual study questionable, however if data for ε2-carriers is treated separately (and either reported or made available upon request) this will at the very least permit future meta-analytic approaches to be conducted on this allele.

It is also becoming increasingly apparent that combinations of genes may operate synergistically to either delay or enhance cognitive recovery post-TBI, and that identification of genes that exert a small but specific effect is an important avenue of research (Weaver et al., 2014). There have been a number of genes that have be implicated in cognitive change post-TBI, and therefore consideration of the interaction between APOE and other genes such as the brain-derived neurotropic factor (BDNF), catechol-O-methyl transferase (COMT), and

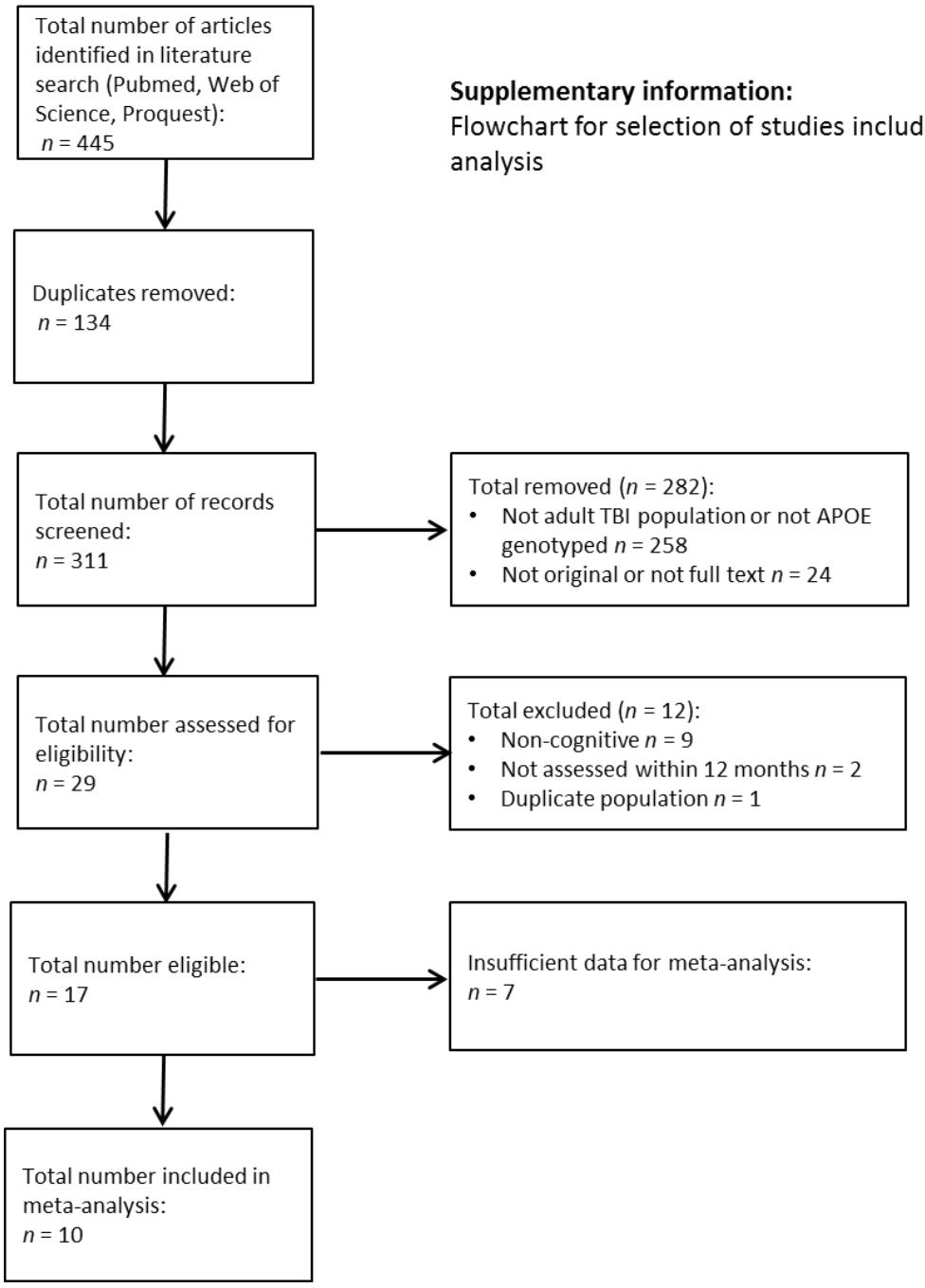
kidney and brain expressed protein (KIBRA) may elucidate the impact of genotype on cognitive recovery (Dardiotis, Grigoriadis, & Hadjigeorgiou, 2012; McAllister, 2010; Wagner et al., 2012; Weaver, Chau, Portelli, & Grafman, 2012).

Finally, there are broader methodological issues that are particularly pertinent in this area of research. The American Psychological Association (2012) has stressed that effect sizes, confidence intervals and complete demographic data should be routinely included in all publications, and full disclosure of results and increased replication studies are also needed to address concerns regarding publication and replication bias (Cumming, 2014). While more recent studies have incorporated some of these elements, given the small effect sizes often reported in the literature to date, and recent concerns regarding publication bias specifically in this field (Dick et al., 2015; Duncan & Keller, 2011), these issues are worth emphasising here. In relation to the concerns regarding sample size, the most apparent solution is increased collaboration and multi-centre research. While the benefits of multi-centre and multi-disciplinary collaborations are well-known, this is particularly apposite for behavioural genetics, where comprehensive, discipline specific knowledge of current advances in genetic research and neuropsychological assessment is crucial for robust investigations. Dick and colleagues (2015) identify a number of associations that provide opportunities to foster such collaborations and could also lead to improved standardisation of measures, and the interested reader is directed to their excellent review and recommendations of the broader issues in cGxE research.

Conclusion.

This is the first meta-analysis exploring the effect of APOE ε4 on cognitive function following TBI, and reveals that APOE ε4 may not be detrimental in terms of post-TBI cognitive function. However, while our meta-analysis provides a more comprehensive picture of the research to date, given the small effects and modest sample size, and recent concerns regarding the prevalence of publication and replication biases in cGxE research (Dick et al., 2015; Duncan & Keller, 2011), our findings must be interpreted cautiously. We suggest that the relationship between APOE genotype and cognitive function following TBI may be better understood with more consistent assessment of injury severity and neuropsychological function, in conjunction with a more nuanced approach to exploring the APOE gene. This would include investigation of moderating factors, consideration of dose-dependent effects APOE ε4 and the possible ameliorative effects of APOE ε2, and exploration of potential interactions between APOE and other genes. While it may not be possible for individual studies to explore these factors, routine reporting of demographic and injury related data, stratified by APOE genotype, or at least the presence/absence of APOE ε4, may clarify the disparate finding to date, and reveal a more complex interaction between APOE and TBI than is currently evident.

Supplementary Material



Supplementary information:
Flowchart for selection of studies included in meta-analysis

Chapter 3: General Methodology

Participants

Participants for the present study were recruited through the Tasmanian Neurotrauma Register (TNTR); a prospective population study of TBI in the Australian state of Tasmania. Located at the Royal Hobart Hospital, Tasmania, the TNTR was established as a joint research unit between the University of Tasmania and the Tasmanian Department of Health and Human Services, with the primary objective of assessing neuropsychological and psychosocial outcomes up to five years following TBI. The TNTR was funded by the Motor Accidents Insurance Board (MAIB), a state-governed scheme which provides compulsory third-party insurance for all Tasmanian registered motor vehicles. The MAIB operates on a common law/no fault basis and provides medical and income compensation and benefits in the event of a motor accident. Ethics approval for the TNTR was obtained from the Tasmanian Human Research Ethics Committee (H0007116), and additional approval for the current studies was provided on the 2nd of September 2010 (see Appendix A for ethics approvals, information sheets and consent forms for both TNTR and APOE study).

All patients aged 16 years and older presenting to the accident and emergency department, or admitted/transferred to the Royal Hobart Hospital (RHH) with a principal or additional diagnosis of TBI, between December 2003 and June 2008 were invited to participate. A total of 2,382 people presented at the RHH within the recruitment period, with 1,226 (51%) agreeing to participate. For those who were unconscious or incapable of giving informed consent at time of admission, next of kin provided initial consent, with the participant consenting on remission of PTA or when appropriate. As the TNTR was a population study there were no inclusion or exclusion criteria, and assessments were undertaken at time of injury, and at 2 weeks, 3, 6, and 12 months, and annually thereafter up to 5 years post injury.

The current research used data obtained at time of injury, 3, 6, and 12 months, and inclusion criteria were as follows:

- The participant must have been aged between 18-75 at time of injury
- No premorbid history of neurological or neurodegenerative disorder
- No previous history of TBI
- No premorbid history of medicated psychological disorders
- Estimated premorbid FSIQ > 70.

Recruitment for the current research occurred between September 2010 and October 2011, with participants being invited to participate in the APOE study when they were contacted for follow-up assessments for the larger TNTR study. A total of 173 participants consented to be in the APOE study, however 3 were unable to be genotyped resulting in a final sample of 170 participants. The APOE genotype distribution is shown in Table 5, and was found to be in Hardy-Weinberg equilibrium [χ^2 (5) = 6.050, p = .30].

Table 5

APOE Genotype Frequencies (N = 170)

APOE Genotype	Frequency
ε4/ε4	5 (2.95%)
ε4/ε3	37 (21.76%)
ε4/ε2	4 (2.35%)
ε3/ε3	106 (62.36%)
ε3/ε2	15 (8.82%)
ε2/ε2	3 (1.76%)

Given the reported opposing effects of APOE ε4 and APOE ε2 (Corder et al., 1994; Suri et al., 2013), participants with a ε2/ε4 genotype were excluded (n = 4), resulting in a total of 166 participants in the APOE study. The demographic and injury-related details for the participants in the current study, and total TNTR sample are provided in Table 6. As can be seen, there were significant differences between the APOE study and the larger TNTR study,

with the only exception being differences in GCS. However, the effect sizes reported are small and indicate that there is little meaningful difference between the two cohorts. Table 7 shows the demographic and injury-related characteristics for the remaining 166 participants, with ANOVA and Chi-square analyses revealing no significant differences between APOE ε4, ε3, and ε2 carriers.

Table 6

Demographic and Injury-Related Data for APOE Study and all TNTR Participants

Characteristic	APOE Study Total (n = 166[a])	TNTR Total (n = 1226[b])	t-test / χ^2	p-value	Cohen's d/ Cramer's V
Sex n (%)					
Male	91 (54.82%)	796 (65%)			
Female	75 (45.18%)	430 (35%)	6.46	.011	0.04
Age at injury (years)					
Mean (SD)	40.09 (16.71)	36.90 (17.81)	2.18	.029	0.18
Premorbid IQ					
Mean (SD)	103.10 (9.78)	97.71 (11.87)	5.39	.001	0.46
Injury Severity (PTA)					
Mild (< 24 hours)	108 (65.06%)	872 (74%)			
Moderate (< 1 week)	32 (19.28%)	187 (16%)			
Severe (> 1 week)	26 (15.66%)	115 (10%)	7.45	.024	0.04
Injury Severity (GCS)					
Mild (13-15)	147 (91.30%)	1,045 (92.72%)			
Moderate (9-12)	5 (3.11%)	29 (2.58%)			
Severe (3-8)	9 (5.59%)	53 (4.70%)	0.41	.813	0.01
Injury Mechanism					
Motor accident	74 (44.58%)	472 (39%)			
Fall	43 (25.90%)	264 (22%)			
Assault	21 (12.65%)	335 (27%)			
Sports	22 (13.25%)	68 (5%)			
Other	6 (3.61%)	87 (7%)	31.04	.001	0.06

[a] APOE data for premorbid IQ *n* = 162; GCS *n* = 161
[b] TNTR data for premorbid IQ *n* = 887; PTA *n* = 1174; GCS *n* = 1127

Table 7

Demographic and Injury-Related Data for APOE Study Stratified by APOE Status

Characteristic	APOE ε4 (ε4/ε4 ε4/ε3) *n* = 42[a]	APOE ε3 (ε3/ε3) *n* = 106[b]	APOE ε2 (ε2/ε2 ε2/ε3) *n* = 18	ANOVA/ χ^2	*p*-value	Partial η^2/ Cramer's V
Sex n (%)						
Male	21 (50%)	58 (54.72%)	12 (66.67%)			
Female	21 (50%)	48 (45.28%)	6 (33.33%)	1.41	.493	0.09
Age at injury (years)						
Mean (SD)	40.45 (16.59)	39.58 (16.52)	42.19 (18.79)	.19	.820	0.01
Premorbid IQ						
Mean (SD)	104.51 (9.69)	102.19 (10.19)	104.90 (6.96)	1.18	.307	0.01
Injury Severity (PTA)						
Mild (< 24 hours)	27 (64.29%)	66 (62.26%)	15 (83.33%)			
Moderate (< 1 week)	8 (19.05%)	21 (19.81%)	3 (16.67%)			
Severe (> 1 week)	7 (16.67%)	19 (17.92%)	0 (0.00%)	4.33	.363	0.11
Injury Severity (GCS)						
Mild (13-15)	36 (87.80%)	93 (91.18%)	18 (100%)			
Moderate (9-12)	2 (4.88%)	3 (2.94%)	0 (0.00%)			
Severe (3-8)	3 (7.32%)	6 (5.88%)	0 (0.00%)	2.43	.657	0.08
Injury Mechanism						
Motor accident	20 (47.62%)	43 (40.57%)	11 (61.11%)			
Fall	13 (30.95%)	29 (27.35%)	1 ((5.55%)			
Assault	5 (11.90%)	13 (12.26%)	3 (16.67%)			
Sports	4 (9.52%)	16 (15.09%)	2 (11.11%)			
Other	0 (0.00%)	5 (4.72%)	1 (5.55%)	8.05	.429	0.16

a APOE ε4 GCS *n* = 41
[b] APOE ε3 premorbid IQ *n* = 102; GCS *n* = 102

Materials

Demographic and screening materials.

Demographic information including age, sex, premorbid medical history, and pharmaceutical/substance use was collected at the initial assessment. Information regarding the TBI was also collected, including injury mechanism, and measures of severity as described below. Premorbid intellectual function was estimated using the National Adult Reading Test (NART). The NART is comprised of 50 phonetically irregular words which participant reads aloud, with the number of correctly pronounced words being converted to an estimate of FSIQ based on the WAIS-III norms (Nelson, 1991). Although there is some evidence that more severe injury is associated with underestimation of FSIQ, performance on the NART has been found to reliably estimate premorbid FSIQ in TBI populations (Morris, Wilson, Dunn, & Teasdale, 2005; Watt & O'Carroll, 1999).

While both PTA and GCS are reported throughout the thesis, injury severity was estimated by length of PTA for all analyses. PTA has been found to be an accurate estimate of injury severity in both mild-to-moderate and severe TBI populations, is considered a more sensitive estimate of injury severity in mild TBI than GCS (Teasdale & Jennett, 1974; van der Naalt, 2001), and is less influenced by factors such as substance use and medical intervention, which may inflate acute GCS scores (Lezak, Howieson, & Loring, 2004; Zuercher et al., 2009). For participants admitted to the RHH (n = 57), PTA was estimated using the Westmead PTA Scale (Shores, Marosszeky, Sandanam, & Batchelor, 1986). PTA for remaining participants (n = 109) was estimated by self-report using the Galveston Orientation and Amnesia Test (GOAT; Levin, Odonnell, & Grossman, 1979). PTA was classified as either mild (less than 24 hours), moderate (1-7 days) or severe (more than 7 days).

Cognitive test battery.

Five neuropsychological tasks were selected, yielding a total of seven measures, as described below. All tasks have been recommended for routine use in assessing post-TBI cognitive function as they are sensitive to neuropsychological impairment following TBI (Atchison et al., 2004; Bagiella et al., 2010; Donders, Tulsky, & Zhu, 2001; Hanks et al., 2008; Henry & Crawford, 2004). These tasks are reported to have minimal practice effects for the time-periods in which assessment was conducted, and/or have alternate versions for repeated administration (Basso, Bornstein, & Lang, 1999; Basso, Carona, Lowery, & Axelrod, 2002; Beglinger et al., 2005).

Adult Memory and Information Processing Battery Information Processing Form A (IP Speed).

The IP Speed task is a subtest from the Adult Memory and Information Processing Battery (Coughlan & Hollows, 1985). It is a speed-based pen and paper task which is a measure of cognitive processing speed which controls for motor speed. The participant first completes a motor speed task, in which they must cross out as many digits as possible in 30 seconds. They are then presented with 105 rows of 5 digits, and have to cross out the second highest number in each row, for as many rows as possible, in 4 minutes. The IP speed score can then be adjusted based on the initial motor speed task (Coughlan & Hollows, 1985). This task has been found to reliably detect information processing deficits in clinical populations (Vlaar & Wade, 2003). Higher values on the IP Speed task indicate better performance.

Trail-making Task B (TMT-B).

The TMT-B is a speed-based paper and pen task in which the participant is required to connect a randomly distributed series of letters and numbers in alternate sequential order (starting with 1-A-2-B…) as quickly as possible. The TMT-B has been reported to measure attention, psychomotor speed, and cognitive flexibility, and is considered to reflect both working memory and executive function (Kortte, Horner, & Windham, 2002; Sanchez-Cubillo et al., 2009; Strauss, Sherman, & Spreen, 2006). Time is measured in seconds, thus a lower score indicates better performance.

Controlled Oral Word Association Task (COWAT).

The COWAT is a speed-based verbal task, in which the participant has 60 seconds to name as many novel words as possible, starting with a particular letter (pronouns and word extensions are not permitted). Three trials are undertaken, each with a different letter. The COWAT is a measure of verbal fluency, and has been shown to indicate executive dysfunction in TBI populations (Henry & Crawford, 2004). Although practice effects for the COWAT have been reported to be minimal (Basso et al., 1999), two parallel versions were administered using either the letters F, A, S, or B, H, T, at alternate assessments (Borkowsk, Benton, & Spreen, 1967). Performance was measured by total number of words across the three trials, thus higher scores indicate better performance.

Digit Span (DS).

The digit span (DS) task from the Wechsler Adult Intelligence Scale III is a measure of short-term memory, attention and immediate verbal recall (Lezak et al., 2004; Wechsler, 1996). For the current study three measures were obtained:

Digit span forwards (DS-F): The DS-F requires the participant to repeat back to the examiner a series of random numbers, in the same order that the examiner presented them. There are two sets for each span, starting with three digits and terminating with nine digits, or when the participant fails two series of equal length. The DS-F score is the longest series of digits correctly recalled, thus higher scores represent better performance.

Digit span backwards (DS-B): The DS-B has an identical structure to DS-F, however the participant must repeat the series of digits in reverse order. Although the DS-B overlaps with the DS-F in terms of function (Bowden, Petrauskas, Bardenhagen, Meade, & Simpson, 2013), due to the additional effort required to reverse the order, the DS-B is considered more challenging and is considered to be more sensitive to neurological impairment than DS-F (Lezak et al., 2004). As for the DS-F, the score is derived from the number of digits correctly recalled and higher values indicate better performance.

Digit Span forward-backwards (DS-FB): Subtracting a participant's total DS-B from the DS-F provides a measure of the discrepancy between of an individual's performance on the DS-F and DS-B. It has been reported that TBI sufferers are likely to have greater divergence between the DS-F and DS-B than normal controls (Lezak et al, 2004). In this measure, lower values indicate better performance.

Letter-Number Sequencing (LNS).

In the letter-number sequencing task (LNS), the examiner reads out a random combination of letters and numbers, and the participant is required to repeat them by providing the letters first, in alphabetical order, then the numbers, in numerical order. Similar to the DS tasks, the LNS is considered to primarily measure attention and short term memory,

however it has also been reported to have a visuo-spatial component (Crowe, 2000). The score for the LNS is the longest sequence correctly recalled; therefore larger values indicate better performance.

Procedure

Participants who were registered in the TNTR study as of 2010 were invited to participate in the current study during routine follow-up contact. Those who agreed completed an additional consent form, and were provided with an additional information sheet (see Appendix A). The neuropsychological tasks used in this research were administered as part of a larger battery of psychosocial, cognitive, and general outcome measures, which took approximately 60 minutes to complete, and participants were invited to take rest-breaks if fatigued. For this study participants were assessed at time of injury, then at 3, 6 and 12 months following TBI, and assessors were blind to APOE status. Table 8 shows the number of participants who completed each task at each time-point, grouped by APOE status.

Table 8

Completion rate for each task at each time-point

	Acute	3 months	6 months	12 months
IP Speed	128 (77.1%)	127 (76.5%)	130 (78.3%)	107 (64.50%)
TMT-B	125 (75.3%)	130 (78.3%)	127 (76.5%)	115 (69.3%)
COWAT	139 (83.7%)	133 (80.1%)	131 (78.9%)	112 (67/5%)
DS-F	139 (83.7%)	134 (80.7%)	132 (79.5%)	115 (69.3%)
DS-B	139 (83.7%)	134 (80.7%)	132 (79.5%)	115 (69.3%)
DS-FB	139 (83.7%)	134 (80.7%)	132 (79.5%)	115 (69.3%)
LNS	134 (80.7%)	134 (80.7%)	130 (78.3%)	113 (68.1%)

As demonstrated in the above table, approximately 80% of participants completed tasks at the acute, 3 and 6 month time periods, and completion rate drops to approximately 69% at 12 months post injury. In chapter 4 this impact of missing data is explored, and is also addressed in the subsequent research chapters (5 and 6).

DNA Analysis

DNA was obtained from saliva samples via buccal swab. Participants rinsed mouth with water, and rubbed the sterile applicator against the buccal mucosa (inner cheek) for 10 seconds, and repeated on opposing side. Applicators were air dried for 30 minutes, then sealed and stored at 4°c until extraction stage. For DNA extraction, 100µl tissue extraction solution ES and 12.5 µl tissue preparation solution was pipetted into tube containing swab. The sample was centrifuged for 5-10 seconds, then swab manually twirled 10-12 times, and excess fluid removed, after which the swab was discarded. Sample was then incubated at room temperature for 10 minutes, and then further incubated at 95°c for 3 minutes. 100µl of neutralisation solution B was then added and sample was again centrifuged for 10 seconds. Samples were then stored at 4°c until PCR could be performed.

Amplification refractory mutations system (ARMS) PCR was used for genotyping, as described by Donohoe, Salomaki, Lehtimaki, Pulkki, and Kairisto (1999). Two reactions were used for each sample (see Appendix B for mixes). Cell extract was added to each reaction, and centrifuged for 10 seconds. 11µl of master mix from reactions A and B were pipetted into a 96 well plate, covered and then PCR was performed using the APOEARMS programme (see Appendix B for description). An agarose (1.5%) gel tray containing SYBR Safe DNA gel stain (5µl/100µl) was submerged in TAE buffer and loaded with 8µl of DNA samples and 4.5 µl of DNA ladder (100 bp, 50 µg/ml). Gel electrophoresis was then performed at 100 volts for 35 minutes (using Bio-Rad Power/Pac 300). Images were captured

using Quantity One (version 4.6) Chemidoc XRS programme and APOE genotypes were identified from images. An example of an electrophoresis image from the study is shown in Figure 7.

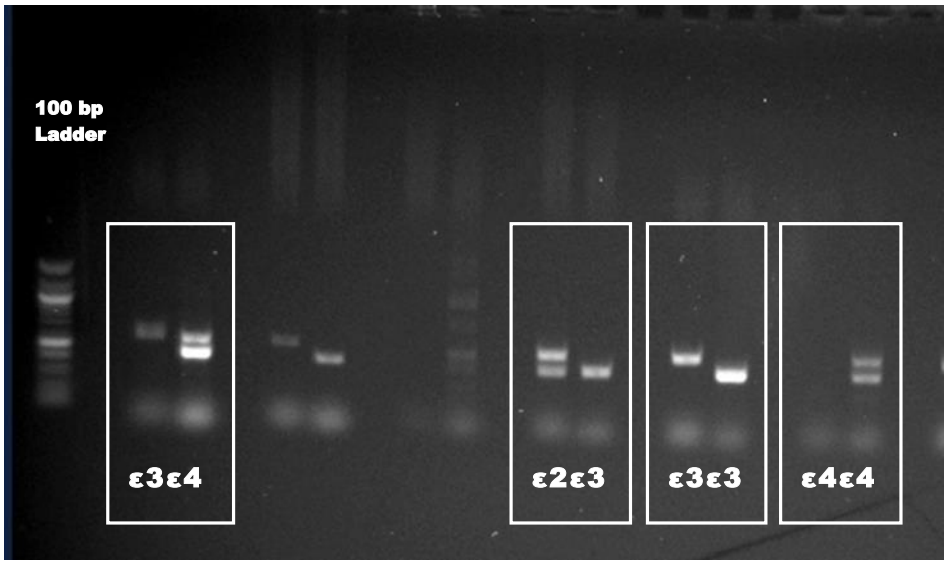


Figure 7. Examples of electrophoresis patterns for APOE genotypes ε3ε4, ε2ε3, ε3ε3 and ε4ε4, using modified ARMS PCR (ε2ε2 and ε2ε4 not shown).

Chapter 4: Missing Data - The Importance and Impact of Missing Data from Clinical Research

This chapter has been published as:

Padgett, C. R., Skilbeck, C. E., & Summers, M. J. (2014). Missing data: The importance and impact of missing data from clinical research. *Brain Impairment, 15*, 1-9.

Abstract

There is compelling evidence that traditional methods used to address the detrimental impacts of missing data are inadequate. Despite this, researchers have been slow to utilise newer statistical approaches known to be more effective. The aim of the current article is to offer a conceptual explanation of the rationale for using newer missing data techniques, with a focus on multiple imputation (MI). To illustrate the relative efficacy of deletion, single imputation and multiple imputation techniques in the clinical setting, 20 cases were randomly selected from a population study investigating the cognitive sequelae of traumatic brain injury (TBI), and 8 out of 20 cases had scores on one variable deleted to simulate a missing dataset. Comparing the parameter estimates obtained by each technique to the known parameters of the complete dataset revealed that MI outperformed deletion and single imputation approaches. It is therefore recommended that more sophisticated techniques such as MI should be considered in clinical research.

Introduction

Missing data (often referred to as missingness) is commonplace in clinical research, where participants may miss appointments, refuse certain tasks, drop out of or die during the course of longitudinal studies. Statistical techniques do not enable the analysis of data in which there are missing cases, consequently different approaches for dealing with the issue of missing values have evolved. Traditionally, the most commonly used approaches for dealing with missing values are deletion or single imputation. Deletion techniques exclude the missing data from analyses, thus statistical analysis is conducted only on complete sets of observed scores. Whilst this may appear to be the most pragmatic approach, ignoring missing data is known to be problematic and has been criticised by methodologists as being one of the most dubious approaches to analysis (Wilkinson & American Psychological Task Force on Statistical Inference, 1999). Imputation techniques involve substituting the missing value with an estimated value. Single imputation techniques provide estimates based on the observed scores on the variable for which the data is missing. The most commonly used single imputation techniques are mean imputation and regression imputation. (Stochastic regression can also be used, however this is rarely employed in clinical research, and is not addressed in this paper.) Mean imputation involves substituting the mean of the observed scores of a given variable for the missing value. Alternatively, where it is possible to conduct a regression analysis, a missing value can be estimated based on a regression equation for the variable.

It has been argued for some time that deletion and single imputation techniques fail to adequately address the impact missing data may have on analysis, with the primary concern being that such approaches do not account for the increased variance that may occur had the actual score(s) been obtained (Allison, 2002; Enders, 2010; Graham, 2012; Schafer & Graham, 2002; Schafer & Olsen, 1998). An additional consequence of deletion techniques is the reduction of statistical power that occurs from the reduction in sample size (Graham, 2009;

McKnight, McKnight, Sidani, & Figueredo, 2007). Therefore missing data has the potential to introduce bias and reduce the integrity of results. With the emergence of increasingly powerful statistical software it is increasingly easy to employ more sophisticated missing data techniques that better compensate for the problems associated with traditional missing data approaches. Two of the more sophisticated approaches are multiple imputation (MI) and maximum likelihood (ML) (Allison, 2002; Baraldi & Enders, 2010; Sinharay, Stern, & Russell, 2001). This report focuses on MI, as it has been suggested this is the most generalisable and flexible missing data technique. MI is robust to breaches of the assumption of normality, is impacted less by the nature of the missing data than other approaches, and performs relatively well even in small samples (Graham, 2012; Graham & Schafer, 1999; Jelicic, Phelps, & Lerner, 2010; McKnight et al., 2007; Sinharay et al., 2001).

Despite repeated recommendations for employing newer missing data techniques such as MI, these approaches are rarely used in clinical/psychological research (Enders, 2010; Jelicic, Phelps, & Lerner, 2009; Roth, 1994; Schafer & Graham, 2002). This is possibly due to a lack of awareness of such approaches, and because the literature regarding these newer approaches can appear daunting for non-statisticians. Furthermore, the detrimental effect that missing data has on analysis is also often underestimated by researchers, thus methods that ameliorate the impact of missing data are not considered necessary. With this in mind, the aim of this report is to offer a conceptual demonstration of how deletion, single imputation and MI compare in estimating missing data when applied in clinical research, in order to demonstrate the impact missing data can have. Firstly, to clarify the effect of missing data on analysis, a description of the nature of missing data, and the limitations of various approaches in addressing missing data, is provided.

Why does missing data matter?

In order to understand why missing data can bias results, it is necessary to briefly explain the nature of missingness itself. The underlying premise of missing data theory is that missingness is often not random in nature. Rubin (1976) proposed that there are three types of missing data. Firstly, data can be missing completely at random (MCAR). MCAR occurs in instances where missing data is not related to the scores on the variable in question, and is also not related to scores on any other variables under analysis. The second category proposed by Rubin is missing at random (MAR). Missing data in this category is not related to scores on the variable with the missing data, however there may be systematic missingness in relation to another variable also being measured. For example, if assessing cognitive function during the acute phase following traumatic brain injury (TBI) it may be that individuals that also experienced orthopaedic injuries when the TBI was sustained would be less likely to complete a design-learning task, due to restricted mobility. In this case, missingness is systematic on one variable (presence of orthopaedic injury/polytrauma) but not on the variable being measured (word-learning), and is therefore MAR. Finally, missing values can be said to be missing not at random (MNAR). This occurs when the missing values are directly related to values of the variable in question. For example, if only participants who are experiencing high levels of depression refuse to complete a questionnaire assessing depression, high scores will be systematically missing on the variable with the missing data.

Missing data therefore has the potential to influence analysis in a number of ways. Clearly, where there is a relatively large amount of missing data and only the complete cases are analysed, the sample size may be seriously reduced. This results in a loss of power, leading to increased risk of Type II errors, whereby meaningful differences are missed (McKnight et al., 2007). An equally pressing concern, long recognised by statisticians but often unrecognised by clinical researchers, is that traditional techniques (deletion, single

imputation) are based on an assumption that missing data occurs randomly, or is MCAR. However, data is often not MCAR, especially in the clinical setting where often there are one or more variables that have the potential to influence which scores are missing. As such, missingness is typically either MAR and MNAR, and therefore bias can occur (Graham, 2009). A further limitation of both deletion and mean and regression imputation is that they only utilise the variance of the observed data set. These methods assume that missing data will fall within the variance range of the existing data set. This is concerning as the missing data is likely to introduce greater variability, and as such ignoring missing data may reduce variance. This attenuates differences where scores are being compared, increasing the likelihood of a Type I error occurring; identifying a significant effect when one is not present (Schafer & Olsen, 1998). Alternately, where correlational analysis is conducted, if mean imputation is used the strength of association becomes weaker, whereas employing regression imputation will strengthen the association, again increasing the risk of misinterpretation of results (Baraldi & Enders, 2010; Schafer & Olsen, 1998). In short, traditional approaches to replacing missing values are based on the frequently erroneous assumption that data is MCAR; increasing the risk of bias and errors in interpretation. Furthermore, the use of traditional techniques such as deletion and single imputation may lead to loss of power, and/or increased risk of Type I or Type II errors.

A conceptual explanation of multiple imputation (MI).

A brief conceptual description of the processes involved in MI is provided below. For more detailed explanations see Enders (2010), Graham (2012), Schafer (1999), and/or Sinharay et al. (2001).

Three separate phases occur in MI; imputation, analysis, and pooling. The imputation phase involves the creation of multiple datasets where missing scores are replaced by scores

obtained from regression equations that are computed from the variable in question and other related variables, as selected by the researcher. Generally, all variables that will be used in final analysis, and any variable expected to interact with the variables under investigation, should be included in the MI (Enders, 2010). Indeed, including additional auxiliary variables (those which may interact with the outcome variable, but may not be of relevance to the research question) is recommended and does not mean that such variables need to be used in later analysis (Enders, 2010). There are a number of algorithms available for use when conducting the imputation phase, however, data augmentation is the most commonly used as it assumes multivariate normality (Enders, 2010; Graham, 2009), and is the focus of the following description.

The imputation phase involves two steps; the imputation step (I-step) and posterior step (P-Step). In the I-step one or more regression equations (based on observed scores of selected variables) are calculated to estimate missing values. To increase variability, an error term derived from the regression is added to each imputed score. From this set of scores, the P-Step calculates new means and co-variances, and again a residual error term is added. The dataset obtained in the P-step can then be used to create another imputed set of scores. In order to ensure that each imputed dataset is independent, a large number of iterations (I-Steps and P-Steps) are run before the next imputed dataset is selected for use in the analysis phase. Because each dataset is based on different regression equations, and generates a different set of parameter estimates, the amount of variance is increased. Thus, MI techniques better account for the impact of unknown variance in the missing data than if a single imputation technique were to be employed. Parenthetically, by comparing the variability of parameters between each of the datasets, the researcher can also estimate the relative impact of the missing data -greater variation between datasets indicating a greater impact of missingness (McKnight et al., 2007). It should also be noted that the number of imputed datasets needed

will vary depending on the amount and nature of the missing data, and predicted effect size (Graham, Olchowski, & Gilreath, 2007; Graham & Schafer, 1999)

When the required number of imputed datasets have been created, the analysis phase is performed, in which the desired analysis is conducted on each dataset separately. In other words, whatever analysis had been planned (regression, ANOVA) can be conducted separately on each of the multiple datasets. Finally, in the pooling phase, the results obtained for each imputed dataset during the analysis phase are combined to obtain a single value reflective of all datasets. For example, means and standard errors could be calculated for each imputed dataset during the analysis phase, and then combined to create a pooled mean and standard error, which can then be reported in the final analysis. The process of pooling varies in complexity, depending on the statistic being calculated. In some situations such as pooling means, the formula for pooling results is the same as for any other data. However, in other situations the process is more complicated, for example pooling standard errors employs a formula in which the variance between and within each imputed dataset is taken into account (for further detail on method of reporting MI results and formulae for pooling see Enders, 2010).

Despite the conceptual nature of the above description, it may appear that MI is a complicated process. However, these steps are automated in many statistical software packages, and therefore MI can be conducted with relative ease. Nevertheless, it should be noted that popular statistical programmes vary in capacity to perform the various stages of MI. For example, whilst SPSS conducts the imputation and analysis phases automatically, it currently doesn't always pool test statistics (for example, if F-tests are performed). Where the test statistic is not calculated, it is possible to create syntax or calculate manually (Enders, 2010; Howell, 2012; Schafer & Olsen, 1998), although clearly this is not ideal and it is hoped that forthcoming versions will soon be more user-friendly. SAS does routinely pool test

statistics, and there are also a number of MI specific programmes such as NORM which also have better functionality.

It is also important to have some understanding of the processes involved in MI as the researcher may need to modify the default settings in some software. Of particular importance are decisions regarding the number of iterations allowed to run between each dataset selected for analysis, and the total number of imputed datasets that should be obtained. For example, in SPSS (version 21) the default number of iterations when choosing the custom setting is 10, whereas it is likely that many more iterations may be necessary, with current recommendations suggesting 100 to 200 iterations are typically required (Baraldi & Enders, 2010; Graham & Schafer, 1999). Furthermore, in SPSS the default number of imputations is 5, and whilst some authors suggest this is acceptable (Sinharay et al., 2001), others suggest that 10 to 20 imputations are required, although this is dependent on the amount of missing data and predicted effect size (Enders, 2010; Graham et al., 2007; Graham & Schafer, 1999).

The current study

Baraldi and Enders (2010) offer a coherent and insightful demonstration that clearly illustrates traditional and newer techniques and their effect. The current study replicates this approach, but rather than create a simulated dataset, the data reported here has been extracted from an existing clinical research project investigating cognitive and psychosocial function following traumatic brain injury (TBI). In order to demonstrate the application of missing data approaches within the clinical research setting a small number of complete cases were randomly selected from the dataset. Parameter estimates (means and standard deviation/error) were calculated, and then some scores were excluded to create a hypothetical missing dataset. The means and standard deviation obtained by deletion, mean imputation,

regression imputation, and MI were then compared to the known mean and standard deviation of the complete dataset in order in order to determine the relative efficacy of each approach.

Method

Description of data set

Data was obtained from the Tasmanian Neurotrauma Register; a population study investigating cognitive and psychosocial recovery following TBI (ethics approval obtained). Individuals with TBI presenting at the Royal Hobart Hospital, Tasmania from 2003-2007 were invited to participate in the Register. At total of 1227 (m = 798 f = 429) individuals consented and underwent a battery of psychosocial and neuropsychological assessments at admission, with follow-up assessments at 3, 6, and 12 months, and annually up to 5 years post injury. For the purpose of the current study, a random draw of 20 participants was selected (m = 11, f = 9), and scores obtained at the initial assessment were used. The injury and demographic variables of the sample are shown in Table 9.

Table 9

Injury and Demographic Characteristics of Participants (n = 20)

	Range	M	SD
Age at injury (years)	21-70	45.56	14.71
Post Traumatic Amnesia (days)	.003 – 9.00	1.11	2.15
Premorbid FSIQ[a]	75 – 121	104.35	11.27

[a]Premorbid full scale IQ was estimated using the National Adult Reading Test (NART).

Materials

Two cognitive measures were selected from the assessment battery. The Controlled Oral Word Association Task (COWAT; Benton & Hamsher, 1989) requires participants to list as many words as possible, starting with a particular letter, within 60 seconds. Three trials, using the letters 'F', 'A', and 'S' were used for all participants, with the total number of words across all three conditions being used in analysis. Coughlan's Information Processing Speed Task (IP Speed; Coughlan & Hollows, 1985) is a measure of processing speed, in which the participant identifies the second highest number in each row of 5 numbers over a set of 105 rows of numbers. The total number of lines in which the correct number is crossed out was the score used in the current analysis.

Procedure

From the 20 selected cases, participants who had attended between June 2005 and June 2006 had their scores on the COWAT deleted, thereby simulating a dataset in which some data was missing due to a hypothetical data entry error. As such, the simulated missing dataset can be said to have data missing at random (MAR), as the likelihood of any given score being missing is not directly related to the variable being measured. Deletion, mean and regression imputation, and MI were then conducted.

Results

SPSS (version 21) was used for all analysis. Means and standard deviations were calculated for the complete set, and then COWAT scores for individuals assessed between June 2005 and June 2006 were excluded, resulting in a total of 8 out of the 20 scores being deleted. Standard approaches to missing data (deletion, and mean and regression imputation) were conducted, and the means and standard deviations obtained using each approach were compared to the known values of the full dataset (Table 10). Scatterplots for the complete dataset (Figure 8), the deletion dataset (Figure 9), mean imputation (Figure 10) and regression imputation (Figure 11), are also provided to enhance illustration.

Table 10

Means and Standard Deviations Obtained Using Deletion, Mean and Regression Imputation Techniques for Hypothetical Missing Dataset, Compared to Complete Dataset

Complete (Observed) Data		Hypothetical Missing COWAT Dataset		
IP Speed	COWAT	Deletion Technique	Mean Imputation	Regression Imputation[a]
38.00	23.00	-	41.67	30.07
67.00	19.00	-	41.67	43.93
60.00	26.00	-	41.67	40.59
59.00	44.00	-	41.67	40.11
52.00	31.00	-	41.67	36.76
38.00	26.00	-	41.67	30.07
72.00	65.00	-	41.67	46.32
58.00	46.00	-	41.67	39.63
50.00	36.00	36.00	36.00	36.00
39.00	18.00	18.00	18.00	18.00
63.00	50.00	50.00	50.00	50.00
42.00	32.00	32.00	32.00	32.00
87.00	54.00	54.00	54.00	54.00
67.00	45.00	45.00	45.00	45.00
86.00	63.00	63.00	63.00	63.00
87.00	47.00	47.00	47.00	47.00
41.00	28.00	28.00	28.00	28.00
68.00	34.00	34.00	34.00	34.00
36.00	44.00	44.00	44.00	44.00
79.00	49.00	49.00	49.00	49.00
Mean	**39.00**	**41.67**	**41.67**	**40.37**
SD	**13.76**	**12.45**	**9.48**	**10.26**

[a] Regression Imputation Equation: $\hat{Y} = 11.989 + 0.478$ (IPSpeed)

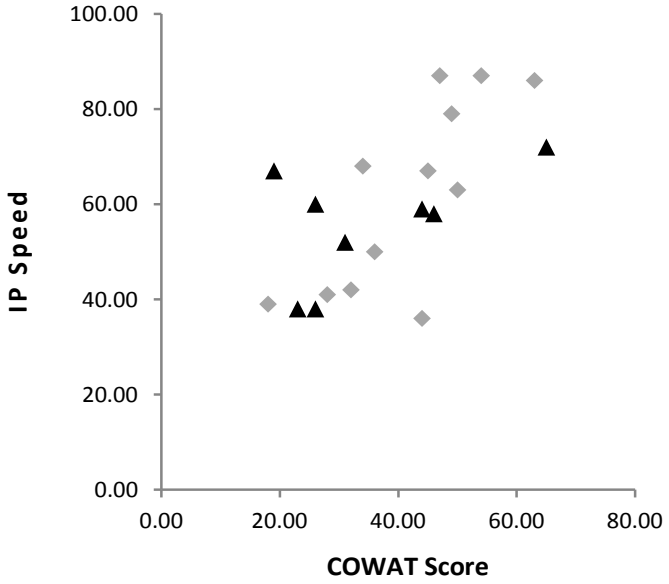


Figure 8. Scatterplot of observed dataset. ▲ Denotes observed data point deleted to create missing data set.

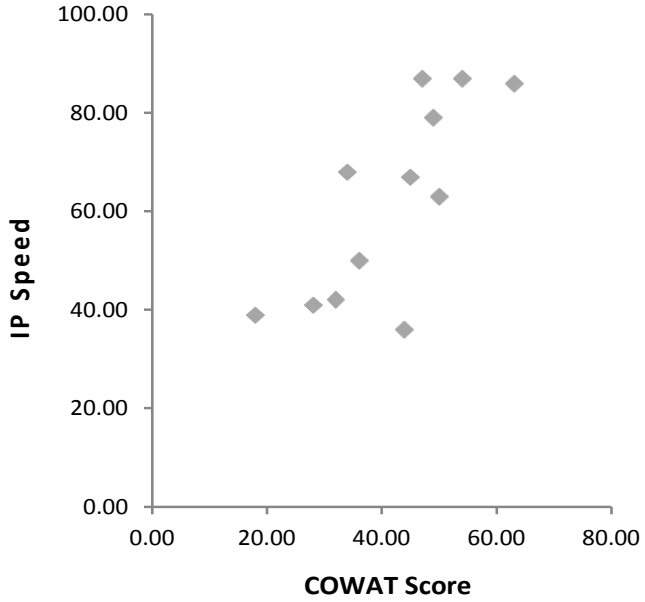


Figure 9. Scatterplot of the hypothetical dataset cases with missing values removed using the deletion technique.

When the deletion technique was used, there was a slight increase in the mean and reduction in variance, as compared to the known parameters (Table 10). The mean obtained from the deletion dataset was then used for the mean imputation approach. Unsurprisingly,

this resulted in the mean remaining the same as for the deletion technique, with a further decrease in variance (Table 10). A bivariate regression analysis was conducted using the IP Speed scores and the COWAT scores from the hypothetical missing dataset (Table 10), with the resulting regression equation being used to imputate the missing COWAT values (see footnote Table 10). The regression imputation mean was closer to the true mean than either the deletion or mean imputation technique. However while the variance was slightly more accurate than variance obtained by mean imputation, it was not as close to the observed SD as when the deletion technique was employed.

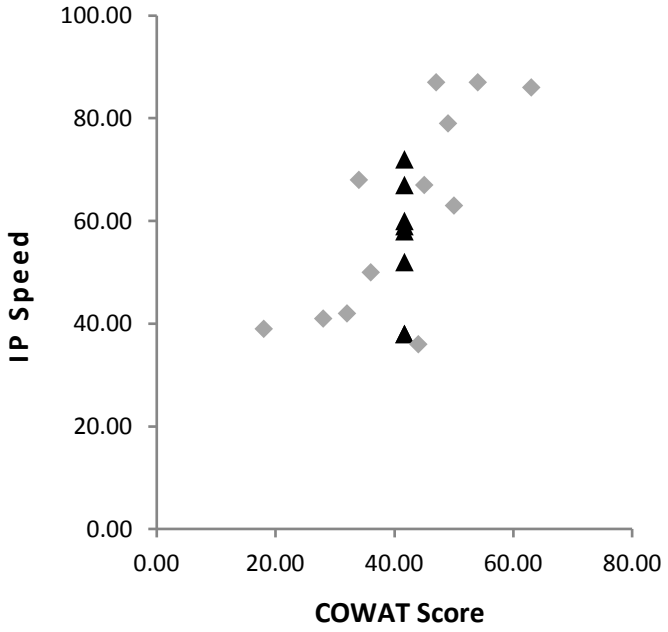


Figure 10. Scatterplot of the hypothetical dataset missing values replaced with mean imputation. ▲ Denotes mean imputed data point.

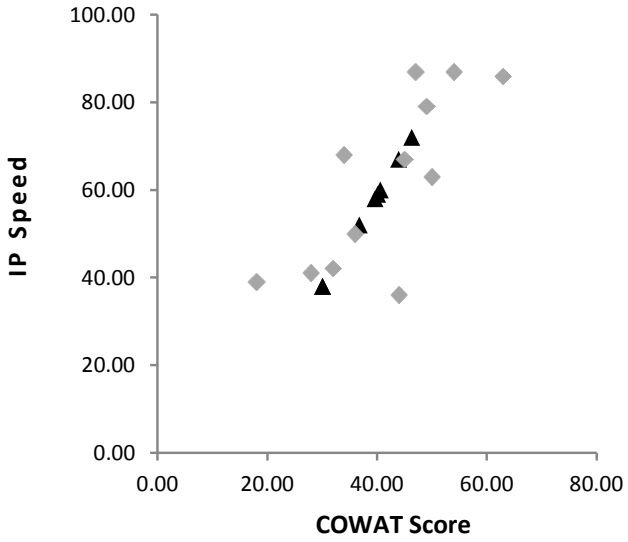


Figure 11. Scatterplot of the hypothetical dataset missing values replaced with regression imputation. ▲ Denotes regression imputed data point.

MI was then conducted, using IP Speed and COWAT scores in the missing dataset. Although it has been recommended that at least 20 imputations be used in most situations (Graham et al., 2007), for the purpose of demonstration a total of 5 imputations were performed (Table 11). In order to increase independence of each imputation, the number of iterations between each imputation was increased from the SPSS default setting 10 to 200, consistent with Baraldi and Enders' demonstration (2010). As is evident in Table 11, each imputation generates different values for a given missing score, thus creating differing means and standard errors (SE). The mean and standard error are pooled for the final analysis. When comparing the pooled mean and standard error to the complete dataset, it is apparent that MI has yielded both a mean and variance very similar to the known parameter estimates, and is closer on both measures than the deletion or single imputation techniques.

Table 11

Comparison of Means and Standard Deviations (SDs) for Complete Dataset, and Hypothetical Missing Datasets Employing Multiple Imputation Techniques

Original Data		**Missing Data**	**Imputed COWAT Scores**					
IP Speed	**COWAT**	**COWAT**	**MI Set 1**	**MI Set 2**	**MI Set 3**	**MI Set 4**	**MI Set 5**	**MI Pooled**
38.00	23.00	-	10.31	33.95	22.94	23.09	50.17	
67.00	19.00	-	33.66	51.92	32.77	26.33	33.12	
60.00	26.00	-	43.81	42.86	38.59	36.45	49.10	
59.00	44.00	-	25.14	52.26	50.86	25.12	34.03	
52.00	31.00	-	35.16	34.57	35.31	0.04	47.08	
38.00	26.00	-	37.51	35.46	25.69	24.30	23.13	
72.00	65.00	-	48.43	32.17	17.29	60.61	27.59	
58.00	46.00	-	16.13	61.45	16.31	43.04	54.99	
50.00	36.00	36.00	36.00	36.00	36.00	36.00	36.00	
39.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	
63.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	
42.00	32.00	32.00	32.00	32.00	32.00	32.00	32.00	
87.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	
67.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	
86.00	63.00	63.00	63.00	63.00	63.00	63.00	63.00	
87.00	47.00	47.00	47.00	47.00	47.00	47.00	47.00	
41.00	28.00	28.00	28.00	28.00	28.00	28.00	28.00	
68.00	34.00	34.00	34.00	34.00	34.00	34.00	34.00	
36.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	
79.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	
Mean	**39.00**	41.67	37.51	42.23	36.98	36.95	40.96	**38.93**
SD/SE	**13.76**	12.45	13.46	11.58	13.23	15.45	11.93	**13.47**[a]

[a] As SPSS does not pool SE this was calculated manually using the formula provided in Baraldi & Enders (2010).

Discussion

In the current study, a random sample of 20 cases was drawn from a clinical research study to compare the means and variance obtained from a complete dataset to an identical dataset, except with missing values. As demonstrated, MI yielded parameter estimates closer to the known estimates than any of the traditional missing data approaches. Each of the deletion and single imputation procedures was limited in its capacity to reflect the complete sample parameters, yielding means and/or variance estimates that were markedly different to the original data set. In contrast, MI was found to provide both a mean and standard error that was close to identical to the known values, and therefore outperformed the deletion or single imputation approaches.

This finding provides similar results to Baraldi and Enders (2010), who used a hypothetical dataset to compare missing data techniques. Indeed, when considering the traditional approaches, in both studies the regression imputation yielded the closest mean, and deletion yielded the closest standard deviation when compared to the complete datasets. An advantage of the current study was that the parameter estimates obtained by each missing data technique were able to be compared to the known parameters of a complete dataset which has been obtained in the clinical setting, thus indicating that missing data techniques such as MI can be applied in this context.

Whilst the above results indicate that MI is an effective method for addressing missing data, it must be acknowledged that the problems associated with missing data cannot be entirely ameliorated by any missing data techniques. MI and other new missing data procedures are useful for data that is MCAR or MAR, however they are less reliable when data is MNAR. Unfortunately, although there are methods to determine if data is MCAR (see Enders, 2010), it is not possible to determine whether data is MAR and MNAR, and to date there is no effective technique for addressing data that is MNAR (Enders, 2010; Graham,

2012). Nevertheless, MI has been found to work relatively well in the MNAR setting (Enders, 2011), and may remain a better alternative than traditional approaches, or even techniques specifically designed to deal with data that is MNAR, as techniques designed for MNAR data rely on untestable and stringent assumptions (Enders, 2010; McKnight et al., 2007; Schafer & Graham, 2002). However, where there are strong violations of the MAR assumption, use of MI or other modern techniques may be questionable.

It should also be noted that even where missingness is MCAR or MAR, MI may not be the most appropriate approach. McKnight and colleagues (2007) state that that small sample sizes may reduce the reliability of MI , however others have suggested this likely to be a reflection of the inherent limitations of small samples, rather than inefficiency of MI per se (Graham, 2009; Graham & Schafer, 1999). Using standard MI in some longitudinal designs and with multilevel data may also be problematic, requiring either the use of an MI algorithm other than data augmentation, or the use of a technique other than MI (Enders, 2010, 2011; Graham, 2012).

A further limitation is that statistical packages still vary in terms of ease of use, especially in relation to pooling parameters and test statistics. Given recent advances, and increased use of modern missing data approaches, it is likely that techniques such as MI will become better integrated into many software packages in the near future. Nevertheless, it is important to be aware of the capacity of a given programme in regards to MI prior to commencing analysis.

An issue not often addressed in the literature to date is the proportion of data missing from a dataset that can be reliably replaced when employing missing data techniques such as MI. McKnight et al. (2007) observe that any such guideline would be arbitrary, and would also depend on the nature of the research. However, it may be advantageous to clarify whether there is a proportion of missingness at which the efficacy of MI is reduced, and

whether there are any considerations specific to applying MI in the clinical research setting, as have been observed for other statistical techniques (Proust-Lima, Dartigues, & Jacqmin-Gadda, 2011). Furthermore, given that clinical researchers may have little experience with such techniques, some provisional guidelines may foster confidence and encourage greater utilisation of these approaches.

It is hoped that by providing this comparison of the various missing data techniques, the benefit of utilising MI in the clinical setting is apparent. It should also be clear that regardless of whether missing data is actively dealt with, it is important to have a sound understanding of the nature and impact of missing data. Ideally, whilst modern missing data approaches can address missingness on a post hoc basis, it is suggested that consideration should be given to the possible factors that may contribute to missing data prior to commencement of research. Any factors that are likely to influence missingness can then be measured, allowing more rigorous analysis. That being said, Graham (2012) states that whilst it is useful to include and understand the key causes of missing data, it is not necessary to include all variables contributing to missing data for MI to be effective. Finally, while beyond the scope of this paper, it should be noted that understanding the nature of missing data can also allow more effective research, both in terms of analysis, and in creating cost-effective studies by utilising a planned missingness design (for further explanation see Enders, 2010 and Graham, 2012).

In conclusion, the nature and detrimental impact of missing data, and the associated limitations of traditional methods of dealing with missingness, are often not fully appreciated. The above findings indicate that modern missing data approaches such as MI outperform traditional approaches by re-introducing the variance that was likely to have occurred should missing scores have been obtained. This minimises the amount of bias caused by missing data, and increases the power to detect meaningful effects. MI and other approaches are now

readily available in many statistical software programs, and are relatively fast and straightforward to use. Whilst there may be some additional effort required to learn new techniques such as MI, it is suggested that the long term benefits make learning these techniques worthwhile, and it is hoped that approaches such as MI will routinely conducted in the clinical research setting.

Chapter 5: Exploring the Effect of the Apolipoprotein Gene on Executive Function, Working Memory and Processing Speed During the Early Recovery Period Following Traumatic Brain Injury.

This chapter published as:

Padgett, C. R., Summers, M. J., McCormack, G. H., Vickers, J. C. & Skilbeck, C. E (in press). Exploring the effect of the APOE gene on executive function, working memory and processing speed during the early recovery period following traumatic brain injury. *Journal of Clinical and Experimental Neuropsychology*.

Abstract

Introduction: There is evidence that the ε4 allele of the APOE gene is detrimental to cognitive function, but results from traumatic brain injury (TBI) populations are mixed. A possible explanation is that APOE ε2 carriers have routinely been incorporated into APOE ε4 and non- ε4 groups, despite APOE ε2 being proposed to have an ameliorative effect. Our primary aim was to investigate the influence of APOE ε4 on cognitive impairment during early recovery following TBI, excluding the potential confound of APOE ε2 possession. A secondary objective was to explore whether APOE ε4 displays more pronounced effects in moderate to severe TBI and to consider the potential post-injury protective influence of the APOE ε2 allele.

Methods: Participants who recently sustained a TBI (Post Traumatic Amnesia > 5 minutes) were assessed on measures of information processing speed, executive function, and working memory upon remission of post-traumatic amnesia. APOE genotype was determined by buccal saliva DNA extraction (APOE ε4 n = 37, APOE ε3 n = 92, APOE ε2 n = 13).

Results: Stepwise multiple regressions were performed to compare APOE ε4 carriers to APOE ε3 homozygotes, with injury severity, age, and estimated premorbid IQ included in the first step. This model was found to significantly predict performance on all tasks, accounting for 17.3% - 24.3% of the variance. When APOE ε4 status was added for the second step, there were no significant changes on any tasks (additional variance <1%). The effect of APOE ε4 in moderate to severe TBI and the effect of APOE ε2 were explored by ANCOVA, with no significant effects revealed.

Conclusions: It is unlikely that APOE genotype influences cognitive function in the initial recovery period following TBI, regardless of injury severity. However, a more nuanced and long-term exploration of the effect of APOE genotype in the TBI population is warranted.

Introduction

Traumatic brain injury (TBI) is a relatively common injury, with some estimates suggesting that 12% of adults will experience a TBI in their lifetime (Frost, Farrer, Primosch, & Hedges, 2013). Those who survive the initial injury can experience deficits to cognitive, emotional and motor function that can be lifelong, with impaired cognitive function being one of the most common and enduring complaints (Draper & Ponsford, 2008; Konrad et al., 2011; Millis et al., 2001). There is emerging evidence that some biological factors may influence cognitive function following TBI. Of growing interest is the Apolipoprotein E (APOE) gene, which synthesises the glycoprotein apolipoprotein e (apoE). ApoE is believed to be one of the key lipid transporters in the brain, and has also been implicated in lipid recycling and clearance (Mahley & Rall, 2000). Lipids, especially cholesterol, are necessary to maintain neurological integrity and to assist in repair processes following TBI, and it has been reported that apoE levels rise dramatically in the initial response to TBI (Adibhatla & Hatcher, 2007). There is also evidence that apoE has antioxidant properties, and may be associated with maintaining the integrity of the blood brain barrier following injury (Horsburgh, McColl, White, & McCulloch, 2003; Methia et al., 2001). Therefore apoE production is thought to be integral in neurological responses to TBI.

Within the human population there are three APOE alleles – APOE ε2 APOE ε3 and APOE ε4, with frequencies of approximately 11%, 72% and 17% respectively (Zannis, Just, & Breslow, 1981). Each allele synthesises structurally different versions of apoE. It has been reported that the protein arising from the APOE ε4 allele has increased misfolding and is less stable than the other two isoforms, and may have reduced efficacy as a result (Mahley & Huang, 2006). Furthermore, there is evidence that possession of the APOE ε4 allele increases risk of developing a number of neurological disorders, most notably Alzheimer's disease (Mahley & Huang, 2006). Given these findings, it has been proposed that possession of the APOE ε4 allele could be associated with greater cognitive impairment post TBI. By far, the

majority of research has focussed on the hypothesised detrimental effect of APOE ε4, but it has also been postulated that APOE ε2 may confer some protective effects in the presence of neuropathology (for a review see Suri, Heise, Trachtenberg, & Mackay, 2013). The APOE ε3 allele is considered neither beneficial nor detrimental (Mahley & Huang, 2012), and is typically used as a reference group to compare APOE ε4 carrier performance against.

Although interest in the relationship between the APOE ε4 allele and cognitive recovery following TBI has grown in recent years, the evidence to date is mixed. When comparing the cognitive performance of APOE ε4 carriers and non-carriers during early stages of recovery (< six months post injury), some researchers report that possession of the APOE ε4 allele is associated with reduced cognitive performance (Anderson et al., 2009; Crawford et al., 2002; Noe, Ferri, Colomer, Moliner, & Chirivella, 2010), whereas others have found no effect of APOE genotype (Liberman, Stewart, Wesnes, & Troncoso, 2002; Müller et al., 2009; Ponsford, Rudzki, Bailey, & Ng, 2007; Shadli, Pieter, Yaacob, & Rashid, 2011). Indeed, findings by Han and colleagues (2007) even suggest that APOE ε4 may have an ameliorative effect following TBI.

There are a number of factors that may contribute to these conflicting reports. A recent review of the literature suggests that APOE ε4 may be associated with poorer cognitive outcomes when TBI is severe (Lawrence, Comper, Hutchison, & Sharma, 2015), but while there is tentative evidence that APOE ε4 may initially be associated with greater cognitive impairment following severe TBI (Liberman et al., 2002; Noe et al., 2010), others have found no such effect in the early recovery period (Ponsford et al., 2007).

There are also methodological limitations inherent in investigations of genetic effects in clinical populations such as TBI which may explain some of the contradictions in research to date. Specifically, the relative infrequency of the APOE ε4 allele has meant that studies are frequently hampered by small sample size for APOE ε4 carriers, with a number of studies having APOE ε4 groups of less than 20 participants (Anderson et al., 2009; Müller et al.,

2009; Shadli et al., 2011). Furthermore, to date existing research investigating short term recovery has included APOE ε2 allele carriers within the APOE ε4 carrier group and/or the non-APOE ε4 carrier group. This may be problematic not only because of the evidence that APOE ε2 confers a protective effect against neurological impairment, but also because APOE ε2 is believed to be dominant over the other two alleles (Corder et al., 1994; Suri, Heise, Trachtenberg, & Mackay, 2013). Given the allelic frequencies of APOE, an estimated 15% of the population would have an APOE ε3/APOE ε2 combination, whereas only 3% of the population are likely to have an APOE ε4/APOE ε2 combination. As such, any protective effect of the APOE ε2 allele would be more likely to impact on the APOE ε3 group, thus having the potential to accentuate differences between APOE ε4 carriers and non-carriers. Due to concerns about the impact of the APOE ε2 allele, more recent long-term investigations have excluded APOE ε2 carriers (Eramudugolla et al., 2014), however, this approach has yet to be applied in studies exploring cognitive function during the initial recovery period following TBI.

The aim of the current study was to determine whether or not APOE ε4 contributes to early (< six months) cognitive impairment, specifically in the domains of executive function, working memory, and processing speed, once other demographic and injury related factors are accounted for. To clarify the effect of APOE ε4, APOE ε2 carriers were treated as a separate group, with the intention that all three alleles could be compared, should there be sufficient APOE ε2 carriers recruited, or allowing a direct comparison of APOE ε4 to the more prevalent APOE ε3 allele if there were insufficient APOE ε2 carriers. The primary hypothesis was that APOE ε4 carriers (individuals who have an ε3/ε4 genotype or are homozygous for the APOE ε4 allele) would have poorer neuropsychological performance than those who are homozygous for APOE ε3, as measured by tests of information processing, executive function and working memory, during the initial recovery phase following TBI. As ancillary hypotheses, we also predicted that the detrimental effect of

APOE ε4 would only occur in moderate to severe TBI, and that APOE ε2 carriers would perform significantly better than either the APOE ε4 or APOE ε3 groups, irrespective of injury severity. Given the infrequency of both APOE ε2 carriers and moderate to severe TBI sufferers in the general population, we expect that sample sizes will be sufficient to permit only exploratory analyses of the two ancillary hypotheses.

Method

Participants

The current investigation into APOE genotype was retrospectively incorporated into a larger longitudinal population study of TBI; the Tasmanian Neurotrauma Register (TNTR). The TNTR operated within the Royal Hobart Hospital, which is the only tertiary referral centre within the state of Tasmania, Australia. Between the years 2003 and 2007, all patients with a diagnosis of TBI, who were admitted as inpatients or seen in the emergency department of the Royal Hobart Hospital, were invited to participate in the TNTR study. (In Australia, a no-fault compensation system operates.) Recruitment and testing protocols were established prior to the commencement of the current study, and participants who had already been recruited into the TNTR were invited to participate in the current study when contacted for their routine follow-up assessments. Those who agreed completed additional informed consent. Inclusion criteria for the current study were: The participant must have experienced more than 5 minutes post-traumatic amnesia (PTA, as measured by either Westmead Scale score, or self-report and the Galveston Orientation and Amnesia Test), been aged between 15-75 years of age at time of injury, have no premorbid history of neurological or neurodegenerative disorders, no previous TBI, no premorbid history of medicated psychological disorders, and premorbid full-scale intelligence quotient (FSIQ) > 70, as estimated by the National Adult Reading Test (NART). Ethics approval was obtained both

for the TNTR study and subsequently for the current study.

Of those who met the inclusion criteria, a total of 173 participants agreed to take part in the APOE study, however three participants were unable to be genotyped. The remaining 170 participants were determined to be in Hardy-Weinberg equilibrium (χ^2 (5) = 6.050, p = .30), and the genotype frequencies are provided the supplementary material section (Table S1). Four participants had a genotype of ε2/ε4, and given the opposing effects of each allele, these participants were also removed from the analysis. A total of 24 participants did not have neuropsychological data for their initial assessment, resulting in a final sample of 142.

Procedure

Following consent, neuropsychological assessment was undertaken as soon as possible following remission from PTA. As neuropsychological assessment occurred prior to DNA sampling, assessors were blind to APOE genotype. DNA samples were collected via buccal swab during routine follow-up assessments and de-identified prior to genotyping. Amplification refractory mutations system polymerase chain reaction (ARMS PCR) was used for genotyping, as described by Donohoe, Salomaki, Lehtimaki, Pulkki, and Kairisto (1999).

The neuropsychological test battery comprised of 5 tasks measuring information processing, executive function and working memory. Information processing speed was measured by the Information Processing (IP) subtest of the Adult Memory and Information Processing Battery, and was adjusted for motor speed (Coughlan & Hollows, 1985). Verbal fluency was measured by the Controlled Oral Word Association Task (COWAT), and cognitive speed and flexibility was assessed by the Trail Making Task B (TMTB), as both tasks have been found to assess executive function (Borkowski, Benton, & Spreen, 1967; Strauss, Sherman, & Spreen, 2006). Scores were obtained by total number of words produced for COWAT and total time taken for TMTB. Working memory was assessed by the Digit

Span (DS) and Letter-Number Sequencing (LNS) subtests of the Wechsler Adult Intelligence Scale (WAIS-III), which were converted to scaled scores (Wechsler, 1996). With the exception of the TMTB and information processing tasks, higher scores are reflective of superior performance. All tests have been found to be reliable and sensitive measures for assessing cognitive recovery in the TBI population, and are predictive of functional outcome following TBI (Henry & Crawford, 2004; Karr, Areshenkoff, & Garcia-Barrera, 2013; Lezak, Howieson, & Loring, 2004; Malojcic, Mubrin, Coric, Susnic, & Spilich, 2008).

Statistical analysis

All analysis was conducted using SPSS version 21. Tests of normality and skewness indicated that scores on the TMTB deviated from normal, however as logarithmic and square root transformations did not significantly alter results the untransformed data was analysed. Chi-Square and *t*-tests were used to compare APOE ε4, ε3, and ε2 groups on demographic and injury factors. Hierarchical multiple regressions were used to compare the performance of APOE ε4 carriers to APOE ε3 homozygotes for each of the five tasks. In the first step, the variables of injury severity, age, and estimated FSIQ were included, as these are consistently found to influence cognitive recovery following TBI (Ponsford, 2013). While we report both Glasgow Coma Scale Score (GCS) and PTA, only PTA was used in the multiple regressions to avoid multicollinearity. PTA was chosen as the predictor as it has been shown to be a more accurate predictor of initial pathology (Schonberger, Ponsford, Reutens, Beare, & O'Sullivan, 2009) and functional outcome (Perrin et al., 2015) than GCS. Injury severity was estimated by PTA and was categorised as mild (<24 hours), moderate (< 1 week) or severe (>1 week), and dummy coded as <1 day versus <1 week, and <1 day versus > 1 week, as has been previously recommended (Lezak et al., 2004), and all levels of injury severity were included in the regression analysis. For the second step, APOE genotype (APOE ε4 carriers versus

APOE ε3 homozygotes) was added.

APOE ε2 carriers were not included in the multiple regressions due to the small number of participants (n = 13); instead ANOVA was considered to be a more robust approach to assess the effect of APOE ε2 as compared to APOE ε4 and ε3 (Howell, 2010), with age, PTA and estimated FSIQ included as covariates when there were significant correlations between these factors and a given task. Similarly, given the modest number of moderate to severe TBI participants (APOE ε4 = 11, APOE ε3 = 31, APOE ε2 = 2), ANCOVAs were conducted to test the additional hypotheses relating to injury severity. With only 2 APOE ε2 participants in the moderate-severe TBI category the APOE ε2 participants could not be analysed. The resultant analysis of moderate-severe TBI included only APOE ε4 and APOE ε3 homozygotes.

Results

The demographic data for the final sample is provided in Table 12. As demonstrated, there were no significant differences between any groups for age, sex, estimated FSIQ, injury severity (PTA or GCS) or injury mechanism. However, while the ANOVA for time between injury and assessment was not significant, Games-Howell post-hoc analysis revealed that the APOE ε2 carriers were assessed significantly closer to time of injury than either the APOE ε4 group (p = .014) or APOE ε3 group (p = .002).

Table 12

Demographic and injury related variables by APOE status

	APOE ε4 (n = 37)	APOE ε3 (n = 92)	APOE ε2 (n = 13)	F-test/χ^2	*p* value
Age (years)					
Mean (SD)	40.62 (17.47)	39.89 (16.89)	41.37 (17.69)	$F(139) = .057$	.945
Sex					
Males	19 (51%)	49 (53%)	9 (69%)		
Females	18 (49%)	43 (47%)	4 (31%)	$\chi^2 (2) = 1.337$	.513
Estimated FSIQ					
Mean (SD)	103.81 (9.84)	102.47 (10.12)	105.88 (7.23)	$F (136) = .802$	.451
Time between injury and assessment					
Mean (SD)	14.84 (13.60)	14.14 (13.75)	7.31 (4.52)		
Median (Range)	11.00 (2-73)	11.00 (1-81)	7.00 (1-17)	$F(139) = 1.720$	.183
Post Traumatic Amnesia (PTA)					
Frequency (%age)					
Mild (< 24 hours)	26 (70.27%)	59 (64.13%)	11 (84.62%)		
Moderate (< 1 week)	7 (18.92%)	19 (20.65%)	2 (15.38%)		
Severe (> 1 week)	4 (10.81%)	14 (15.22%)	0 (0.0%)	$\chi^2 (4) = 3.154$	.532
Glasgow Coma Scale Score (GCS)					
Frequency (%age)					
Mild (13-15)	34 (83.3%)	82 (86.8%)	12 (92.3%)		
Moderate (9-12)	1 (4.8%)	3 (2.8%)	0 (0.0%)		
Severe (3-8)	1 (7.1%)	4 (5.7%)	0 (0.0%)		
Not Recorded	1 (4.8%)	3 (4.7%)	1 (7.7%)	$\chi^2 (6) = 1.899$	.929
Injury Mechanism (%age)					
Motor vehicle accident	16 (43.2%)	37 (40.2%)	7 (53.8%)		
Fall	14 (37.9%)	29 (31.5%)	1 (7.7%)		
Assault	5 (13.5%)	12 (13.1%)	3 (23.1%)		
Sports	1 (2.7%)	12 (13.1%)	1 (7.7%)		
Other	1 (2.7%)	2 (2.1%)	1 (7.7%)	$\chi^2 (8) = 8.347$	.401

Hierarchical multiple regression analysis

Descriptive data (stratified by injury severity) for APOE ε4 and APOE ε3 groups is provided in Table 14 in the supplementary section of this chapter. Hierarchical multiple regressions were conducted for each task, comparing APOE ε4 carriers to the APOE ε3 homozygotes. For the first step, the predictor variables of PTA, age and estimated FSIQ were included. Genotype (APOE ε4 vs APOE ε3) was added in the second step. The results for the multiple regressions are shown in Table 13. As can be seen, the first model was predictive of outcome for IP speed, $F(4,108)= 5.984$, $p=.001$ (18.1% of variance); TMTB $F(4,106)= 7.116$, $p = .001$ (21.2% of variance); COWAT $F(4,118)= 6.164$, $p=.001$ (17.3% of variance); DS, $F(4,118)=9.473$, $p=.001$ (24.3% of variance); and LNS, $F(4,113)=6.563$, p=.001 (18.9% of the variance). As can also be seen in Table 13, the addition of APOE genotype in the second step did not significantly improve any of the models, with an additional contribution of less than 1% for each task.

Table 13

Hierarchical multiple regression data for IP speed, COWAT, TMTB, DS and LNS (APOE ε4 carriers vs APOE ε3 homozygotes)

		Unstandardized coefficient		*Standardised coefficient*			
Task	Variable	B	SE	β	p value	R^2	ΔR^2
IP Speed							
Step 1	Age	-.355	.115	-.291	.002	.181	.181*
	Estimated FSIQ	.467	.195	.228	.018		
	PTA (<1 day vs < 1 week)	-10.171	4.712	-.198	.033		
	PTA (<1 day vs > 1 week)	-13.885	5.805	-.215	.018		
Step 2	Genotype	6.212	4.093	.132	.132	.199	.017
COWAT							
Step 1	Age	.270	.149	.164	.072	.173	.173*
	Estimated FSIQ	.563	.257	.201	.030		
	PTA (<1 day vs < 1 week)	-10.719	6.051	-.153	.079		
	PTA (<1 day vs > 1 week)	-19.663	7.255	-.234	.008		
Step 2	Genotype	-6.375	5.207	-.102	.223	.183	.010
TMTB							
Step 1	Age	1.023	.201	.478	.001	.212	.212*
	Estimated FSIQ	-.623	.335	-.176	.066		
	PTA (<1 day vs < 1 week)	2.203	8.054	.024	.785		
	PTA (<1 day vs > 1 week)	15.066	10.293	.129	.146		
Step2	Genotype	-.563	6.734	-.007	.933	.212	.001

Continued overleaf…

Task	Variable	*Unstandardized coefficient*		*Standardised coefficient*	p value	R^2	ΔR^2
		B	SE	β			
DS							
Step1	Age	-.005	.013	-.034	.696	.243	.243*
	Estimated FSIQ	.119	.022	.470	.001		
	PTA (<1 day vs < 1 week)	-.618	.525	-.097	.241		
	PTA (<1 day vs > 1 week)	-.704	.612	-.095	.253		
Step2	Genotype	.517	.455	.091	.258	.251	.008
LNS							
Step 1	Age	-.010	.014	-.061	.508	.189	.189*
	Estimated FSIQ	.098	.025	.368	.001		
	PTA (<1 day vs < 1 week)	-.315	.610	-.045	.607		
	PTA (<1 day vs > 1 week)	-1.757	.678	-.225	.011		
Step 2	Genotype	.481	.502	.081	.340	.195	.007

* p <.05

Ancillary analyses for moderate to severe TBI participants and for APOE ε2 carriers.

In order to explore whether APOE ε4 is increasingly detrimental to cognitive performance as TBI severity increases, ANCOVAs (with age, PTA and estimated FSIQ included as covariates when significant correlations were present) were conducted comparing the performance of APOE ε4 and APOE ε3 participants who were classified as sustaining a moderate to severe TBI. These revealed no significant differences between the performance of APOE ε4 carriers and APOE ε3 carriers on any tasks. ANCOVAs were also conducted on all participants, including APOE ε2 carriers, to explore whether APOE ε2 carriers displayed superior performance to the other two groups. This analysis also failed to find any significant differences between APOE ε2, ε3, and ε4 carriers on any tasks. The descriptive statistics, F-values and associated effects sizes for these analyses are provided in Table 14 and 15 which are in the supplementary section following this chapter's discussion section.

Discussion

The hypothesis that possession of the APOE ε4 allele would be predictive of reduced executive function, working memory, or processing speed during the early recovery phase following TBI was not supported. Adding APOE genotype to a multiple regression model did not improve the predictive ability of the model for any of the neuropsychological tasks after variance explained by age, estimated premorbid IQ, and severity of injury had been accounted for. Although some previous studies have found evidence of an APOE ε4 associated cognitive decline during the early recovery period following TBI (Anderson et al., 2009; Crawford et al., 2002; Noe et al., 2010), the current finding aligns with previous research in which no significant effect of APOE ε4 on cognitive outcome was found during short term recovery (Liberman et al., 2002; Müller et al., 2009; Ponsford et al., 2007; Shadli

et al., 2011), suggesting that possession of the APOE ε4 allele does not lead to greater cognitive impairment following TBI.

Additional analysis of participants with moderate to severe TBI reveal no detrimental effect on cognitive performance for APOE ε4 carriers as compared to APOE ε3 homozygotes. While this contradicts the recent review by Lawrence and colleagues (2015), who observed that poorer outcomes for APOE ε4 carriers were more frequently reported in studies in which severe TBI was investigated, it must be stressed that our analysis was highly exploratory due to the small sample, and as such requires cautious interpretation. We also found that APOE ε2 carriers did not outperform the other two groups for any of the tasks, suggesting that APOE ε2 may not exert a protective influence following TBI. To our knowledge, this is the first report on the effect of APOE ε2 on cognitive function during the early recovery period following TBI. While our findings may indicate that the inclusion of APOE ε2 into APOE ε4 non-carrier groups has not accentuated the differences between APOE ε4 carriers in previous reports, the tentative nature of our findings must be emphasised. Given the evidence that APOE ε2 plays both a dominant and protective role in neurological integrity and cognitive function in non-TBI populations (Suri et al., 2013), future investigation with larger samples is needed to explore the role of the APOE ε2 allele in cognitive function following TBI.

At the time of submission, this study has the advantage of containing the largest sample of APOE ε4 carriers of any study in which neuropsychological tasks have been used during the early recovery period. However, there are some limitations to the current study. Most of our participants had sustained mild injuries (PTA < 24 hrs for 67.60% of the total sample), and although we explored the effect of injury severity, the small sample size for that analysis limits the robustness of the results. We also note that had we used GCS as our measure of injury severity, 90% of participants would have been classified as sustaining a

mild TBI. This highlights the discrepancy that can occur in estimating injury severity, depending on the measure used, as has been observed by others researchers (Sherer, Struchen, Yablon, Wang, & Nick, 2008). We chose to use PTA as the primary measure of severity as GCS can be confounded by a number of factors (Zuercher, Ummenhofer, Baltussen, & Walder, 2009), and PTA has been reported to be more closely associated with measures of pathology and other functional outcomes following TBI than GCS (Perrin et al., 2015). Nonetheless, GCS remains a popular measure of severity, and we acknowledge that this reduces the ability to compare our study and previous literature using GCS. Additionally, while participants were assessed as soon as possible following injury, it is also possible that those with mild TBI had recovered prior to assessment. However, time between injury and assessment was associated with severity of injury, insofar as assessments were not undertaken until PTA was resolved, and thus most mild TBI sufferers were seen relatively soon after injury.

Animal and cellular studies also indicate that both levels of sex hormones and age influence apoE expression and the neurological response to apoE (Horsburgh, Macrae, & Carswell, 2002; Mannix et al., 2011; Struble, Nathan, Cady, Cheng, & McAsey, 2007). Sex and age frequencies were equivalent between our APOE groups, therefore it is unlikely that there were any systematic between group effects, but we did not investigate whether there was any sex or age related differences within the APOE ε4 carrier group. Furthermore, our sample was predominantly comprised of young and middle aged adults (approximately 70% being <50 years of age), and it has been well established that older age is related with poorer outcomes following TBI (Ponsford, 2013). APOE ε4 is also associated with age-related neurological disorders such as Alzheimer's disease (Mahley & Huang, 2006), in which cognitive impairment is a defining feature. Thus, it is possible that when elderly APOE ε4 carriers sustain a TBI, the effects of TBI and APOE ε4 may work synergistically to result in

poorer cognitive outcomes, or that when a TBI is sustained by APOE ε4 carriers prior to late-adulthood, this augments disease-related pathology later in life, which would be unlikely to be detected in our sample.

There is also tentative evidence that APOE may be expressed via an antagonistic pleiotropic mechanism, whereby the APOE ε4 allele confers a protective effect before and during the reproductive life phase, but exerts a detrimental effect in the post-reproductive phase (Han & Bondi, 2008; Jochemsen, Muller, van der Graaf, & Geerlings, 2012; Rusted et al., 2013). If this mechanism is demonstrated for APOE, it is possible that only APOE ε4 carriers who sustain a TBI in later life will demonstrate poorer outcomes. Such a mechanism would align with evidence from Han and colleagues (2007), who found in a young adult cohort that APOE ε4 carriers performed better than non-carriers on a broad range of neuropsychological tasks following TBI. It is also noteworthy that Ponsford and colleagues (2011) observed that female APOE ε4 carriers over the age of 55 had poorer long term functional outcomes than age-equivalent APOE ε4 males, and non APOE ε4 females, suggesting a possible interaction between age, sex and APOE. Others have not found such an effect, with Friedman et al. (1999) reporting no age-related differences in general cognitive function for APOE ε4 carriers, and a study Rapoport and coworkers (2008) revealing no differences between older (> 50 years) TBI sufferers on tasks of learning, memory, or executive function, based on APOE ε4 status. Furthermore, Teasdale, Murray and Nicoll (2005) reported that APOE ε4 was more detrimental in terms of general functional outcome for young APOE ε4 carriers following TBI than for older APOE ε4 carriers. These contradictory findings suggest that further investigation is warranted to clarify whether age moderates the relationship between APOE and outcome following TBI.

It is also possible that the effect of APOE ε4 is domain specific, and that our tasks did not capture some functional differences. Specifically, both Crawford et al. (2002) and

Anderson et al. (2009) reported that verbal learning and memory functions were impaired during initial recovery in their cohorts, whereas performance on measures of executive function, psychomotor speed and visuo-spatial ability remained similar for both APOE ε4 carriers and non-carriers. Although DS has been argued to measure elements of short term verbal memory (Lezak et al., 2004), we did not employ any verbal learning tasks, as our study was part of a larger pre-existing longitudinal study which did not include these measures. Further investigations using measures of verbal learning and memory in APOE ε4, ε3 and ε2 carriers may therefore prove enlightening.

Although this result bolsters the evidence that there is no effect of APOE ε4 on post-TBI cognitive function, further research exploring later cognitive recovery, and more nuanced explorations of the APOE gene are warranted. For example, some studies have found APOE ε4 to be deleterious to cognitive recovery during the 6-12 month recovery period (Ariza et al., 2006; Friedman et al., 1999; Müller et al., 2009). This may suggest that any detrimental effect of APOE ε4 is initially masked by other acute neurobiological responses to injury, but may be revealed in later recovery. It is also becoming increasingly evident that genetic expression is frequently impacted by biological and environmental factors, and there have been calls to consider such interactions when investigating the effect of APOE in the TBI population (Weaver et al., 2014).

In conclusion, we did not find any evidence that APOE ε4 is associated with impaired in cognitive function during the acute recovery period following TBI, or that any detrimental effect of APOE ε4 is pronounced following more severe TBI. However, exploration of later time-points, using a broader range of neuropsychological tasks, and investigation of the possible moderating effect of sex and/or age on APOE ε4, may clarify the role of the APOE gene in post-TBI cognitive outcomes. We also did not find that APOE ε2 was associated with any significant differences in function, but given the tentative nature of these findings,

future research into the effect of APOE genotype would nonetheless benefit by employing a more considered approach to APOE ε2, with APOE ε2 carriers being either excluded, or treated as a separate group, when investigating the impact of APOE in cognitive function after TBI, and in other clinical and non-clinical populations.

Supplementary Material

Table 14

Descriptive data and ANCOVAs for performance by moderate to severe TBI (APOE ε4 and APOE ε3 only)

Task	*APOE ε4*		*APOE ε3*		*ANOVA*		
	n	*Mean (SD)*	*n*	*Mean (SD)*	*F*	*p*	η_p^2
IP Speed[a]	11	60.91 (12.99)	28	56.18 (16.61)	.512	.479	.014
COWAT	11	28.82 (15.28)	30	24.67 (9.86)	.919	.344	.024
TMTB[a]	10	105.00 (31.17)	26	97.77 (44.55)	.169	.683	.005
DS (scale)	11	8.36 (2.34)	31	8.87 (2.25)	.677	.416	.017
LNS (scale)	10	7.90 (2.47)	27	9.07 (2.28)	2.008	.166	.057

[a] A lower score on these tasks is indicative of better performance.

Table 15

Descriptive data and ANCOVAs for performance on all tasks, grouped by APOE genotype

Task	*APOE ε4*		*APOE ε3*		*APOE ε2*		*ANOVA*		
	n	*Mean (SD)*	*n*	*Mean (SD)*	*n*	*Mean (SD)*	*F*	*p*	η_p^2
IP Speed[a]	32	63.31 (19.47)	83	68.52 (22.20)	13	66.92 (12.73)	1.531	.220	.025
COWAT	36	34.17 (13.72)	90	31.22 (11.54)	13	37.46 (8.86)	.648	.525	.010
TMTB[a]	36	90.06 (32.31)	78	91.76 (41.07)	11	79.36 (325.80)	.873	.420	.014
DS (scale)	35	9.20 (2.29)	91	9.37 (2.76)	13	10.00 (2.61)	.661	.518	.010
LNS (scale)	34	9.38 (2.67)	87	9.57 (2.75)	13	10.00 (2.48)	.415	.661	.007

[a] A lower score on these tasks is indicative of better performance.

Chapter 6: Does Apolipoprotein ε4 Interact with Age or Sex in Post-Traumatic Brain Injury Cognitive Function?

This chapter submitted for publication (under review) as:

Padgett, C. R., Summers, M. J., Honan, C. A., McCormack, G. H., Vickers, J. C., & Skilbeck, C. E. (under review). Does Apolipoprotein ε4 interact with age or sex in cognitive function after traumatic brain injury? *Brain [BRAIN-2016-00203]*.

Abstract

Possession of the APOE ε4 allele has been suggested to lead to poorer cognitive function following traumatic brain injury relative to other allelic versions of the APOE gene, however, findings to date have proven equivocal. For conditions other than traumatic brain injury, there is some evidence that the effect of APOE ε4 is influenced by age and/or sex, which may account for the mixed results in traumatic brain injury literature, but these relationships are yet to be systematically explored in the context of post-injury cognition. The aim of this study was to investigate the impact of APOE ε4 on cognitive function following traumatic brain injury, and to explore the influence of age and sex on the impact of APOE ε4 versus the most common APOE allele; ε3. Participants diagnosed with traumatic brain injury were genotyped to determine APOE status (APOE ε4 n = 30, APOE ε3 n = 77, APOE ε2 n = 12) and were assessed using a battery of cognitive tasks measuring executive function, working memory, and processing speed at 3, 6 and 12 months post-injury. APOE ε2 carriers were excluded due to small sample and the possibility that APOE ε2 is both protective and dominant over APOE ε4 and ε3, and the cognitive function of APOE ε4 and APOE ε3 carriers was compared using a mixed model approach. It was revealed that APOE ε4 carriers performed worse than the APOE ε3 group on only two of seven tasks (Trail Making Task B at 6 months, and the Controlled Oral Word Association Task), and therefore possession of APOE ε4 did not appear to systematically impair cognitive function. To explore the relationship between APOE ε4 and age, participants were categorised as young (18-50 years) or older (51-70 years) adults, and the interaction between APOE ε4 and sex was also investigated. There was no evidence of interactions between age and APOE ε4, or sex and APOE ε4. Our findings indicate that the APOE gene is unlikely to significantly impact on cognitive function following traumatic brain injury, and that neither age nor sex interact with APOE ε4 in this population. While the injury and demographic characteristics

of our sample were reflective of the broader traumatic brain injury population, further examination of these relationships in moderate to severe samples may be warranted.

Keywords: traumatic brain injury, Apolipoprotein E, executive function, memory, gene-environment interaction.

Introduction

One of the most common and enduring outcomes following traumatic brain injury (TBI) is impaired cognition (Draper & Ponsford, 2008), and there has been increased interest in exploring the utility of a number of biomarkers in predicting cognitive outcome. One such marker is the APOE gene. The APOE gene synthesises apolipoprotein e (apoE) which is believed to be an essential factor in recovery following TBI due to its roles in lipid transport, recycling, and clearance (Mahley & Rall, 2000). The APOE gene has three alleles; APOE ε2, APOE ε3 and APOE ε4, with estimated population frequencies of 11%, 72%, and 17% respectively (Zannis, Just, & Breslow, 1981). To date, research has focussed on the hypothesis that the APOE ε4 allele may be deleterious, as it has been reported to synthesise an isoform of apoE that is less stable than the apoE ε2 or apoE ε3 isoforms, has been found to increase risk of developing Alzheimer's disease (Corder et al., 1993; Kim, Basak, & Holtzman, 2009), and may be associated with poorer outcomes in other neuropathological events (Maxwell et al., 2011; Verghese, Castellano, & Holtzman, 2011). Possession of APOE ε4 has also been demonstrated to be associated with both earlier accumulation and increased levels of amyloid pathology, even in the absence of disease processes (Jansen et al., 2015), with animal studies indicating that the increased amyloid burden is associated with poorer cognitive function (Yin et al., 2014). Given the evidence that APOE ε4 may reduce neurological integrity, it has been proposed that TBI sufferers who possess the APOE ε4 allele may experience greater cognitive impairment or delayed recovery following injury.

Although animal studies consistently demonstrate that APOE ε4 leads to poorer neurological integrity and cognitive function post injury (Horsburgh, McCarron, White, & Nicoll, 2000; Sabo et al., 2000; White, Nicoll, Roses, & Horsburgh, 2001), the evidence is less clear in human TBI research. A number of researchers have reported that APOE ε4 carriers experience greater cognitive impairment (Anderson et al., 2009; Ariza et al., 2006;

Crawford et al., 2002; Friedman et al., 1999; Noe, Ferri, Colomer, Moliner, & Chirivella, 2010; Sundstrom et al., 2004), and reduced recovery trajectories (Müller et al., 2009) than non-carriers during the first 12 months of recovery, with Eramudugolla et al. (2014) reporting that APOE ε4 is also associated with poorer long-term cognitive outcomes. Conversely, others have found no differences in cognitive function either in the first 12 months following injury (Chamelian, Reis, & Feinstein, 2004; Hodgkinson, Gillett, & Simpson, 2009; Liberman, Stewart, Wesnes, & Troncoso, 2002; Ponsford, Rudzki, Bailey, & Ng, 2007; Pruthi et al., 2010; Shadli, Pieter, Yaacob, & Rashid, 2011), or in long-term recovery (Ashman et al., 2008; Isoniemi, Tenovuo, Portin, Himanen, & Kairisto, 2006; Rapoport et al., 2008; Teasdale, Jorgensen, Ripa, Nielsen, & Christensen, 2000). Indeed, there are some reports that APOE ε4 may be associated with reduced cognitive impairment after TBI (Han et al., 2007). Thus, the findings to date are mixed, and consequently the establishment of the nature of the relationship between APOE ε4 and post-TBI cognition has proven difficult.

It has been argued that interactions between biological and environmental factors need more consideration when investigating genetic effects in relation to psychological function, and specifically in TBI populations (Dick et al., 2015; Weaver et al., 2014). A failure to explore such interactions may explain the equivocal findings in prior research relating to the impact of APOE ε4 in post-TBI cognition. Notably, there is emerging evidence that age and/or sex might influence the expression of APOE ε4, both in terms of cognitive function and risk for developing neurological disorders (Beydoun et al., 2012; Farrer et al., 1997; Ghebremedhin et al., 2001; Jochemsen, Muller, van der Graaf, & Geerlings, 2012). Despite this, there has been minimal exploration of these factors in relation to cognitive function following TBI.

There is some evidence from non-TBI literature that there is an interaction between APOE status and age. For example, the reported relationship between APOE ε4 and

Alzheimer's disease (which typically commences in late adulthood) has led some authors to propose that APOE operates via an antagonistic pleiotropic mechanism, whereby APOE ε4 confers some benefits before and during the reproductive life phase, but becomes deleterious during the post-reproductive life phase (Carter & Nguyen, 2011; Han & Bondi, 2008; Leroi et al., 2005; Tuminello & Han, 2011). In healthy human populations, some researchers have reported that young adult APOE ε4 carriers have superior cognitive function when compared to non-carriers (Alexander et al., 2007b; Marchant, King, Tabet, & Rusted, 2010; Mondadori et al., 2007; Puttonen, Elovainio, Kivimaki, Lehtimaki, & Keltikangas-Jarvinen, 2003; Rusted et al., 2013), and others have reported that APOE ε4 carriers have poorer cognitive function than non-ε4 carriers in late adulthood (Greenwood, Espeseth, Lin, Reinvang, & Parasuraman, 2014; Jochemsen et al., 2012; Shin et al., 2014), providing some support for an antagonistic pleiotropic mechanism. However others have found no differences, either in young or old aged cohorts (Bunce, Anstey, Burns, Christensen, & Easteal, 2011; Deary et al., 2003; Ihle, Bunce, & Kliegel, 2012; Jorm et al., 2007), thus this effect remains speculative.

In the few studies which have considered the interaction between age and APOE status in the context of TBI, there is tentative support for an antagonistic pleiotropic effect. Animal models of TBI suggest that older APOE ε4 carriers may experience greater cognitive impairment following brain injury than younger APOE ε4 carriers, or young and old non-carriers (Mannix et al., 2011), and in their investigation of cognitive performance following TBI in children aged 8-15 years, Moran and colleagues (2009) reported that APOE ε4 carriers performed better than non-APOE ε4 carriers on a visual motor integration task. Han et al. (2007) also found that young adult (mean age 22 years old) APOE ε4 carriers performed significantly better than non-carriers on tasks of attention, executive function, and episodic memory, one month following mild to moderate TBI.

Conversly, Eramudugolla and colleagues (2014) reported that APOE ε4 carriers had poorer episodic memory in early adulthood, and poorer reaction time in middle-aged adulthood in TBI. In addition, they also found that if the brain injury was sustained during childhood, no genotypic differences in cognitive function were apparent by late adulthood. Also in contradiction to the antagonistic pleiotropic effect, Friedman et al. (1999) found that while APOE ε4 was predictive of a poorer cognitive performance 6-8 months following injury, an interaction between age and APOE status was not found. Lastly, when comparing the cognitive function of APOE ε4 carriers and non-carriers aged 50 years and older, at 12 and 24 months following mild to moderate TBI, Rapoport and co-workers (2008) also reported no significant differences. In short, the role that age may play in the relationship between APOE e4 and cognitive function is not clear.

It is also possible sex differences interact with APOE status. Animal and *in vitro* studies have demonstrated that the expression of APOE is moderated by sex hormones (Koutseff, Mittelhaeuser, Essabri, Auwerx, & Meziane, 2014; Struble, Nathan, Cady, Cheng, & McAsey, 2007), and that the neuro-protective effects of apoE are enhanced by higher levels of oestrogen (Struble et al., 2007), with evidence that this relationship is less efficacious for the apoE ε4 isoform (Lambert, Coyle, & Lendon, 2004; Raber et al., 1998) and that androgens may be protective in the presence of APOE ε4 (Raber, Bongers, LeFevour, Buttini, & Mucke, 2002). As a result, females may be more vulnerable to an APOE ε4 associated detrimental effect, and indeed studies in human populations indicate that female APOE ε4 carriers compared to males and non-carrying females may be at greater risk of cognitive impairment (Bartres-Faz et al., 2002; Beydoun et al., 2012; Mortensen & Hogh, 2001), reduced hippocampal connectivity and volume (Fleisher et al., 2005; Heise et al., 2014) and development of Alzheimer's disease (Altmann, Tian, Henderson, Greicius, & Initi, 2014; Farrer et al., 1997). However, it must be noted that the aforementioned research was

undertaken in the context of risk of developing Alzheimer's disease, and only recruited older participants. In the only study we identified exploring this relationship in young adults, Yu, Lin, Chen, Hong, and Tsai (2000) found young APOE ε4 females had improved performance intelligence scores as compared to female non-carriers. A limitation to the interpretability of this study is that males were not included, and although there were significant differences between groups, the mean scores for both fell within the normal intelligence range and would likely lack clinical relevance. In summary, the moderating effect of sex on the relationship between cognitive function and APOE e4 is unknown.

To date, there is no published literature examining the potential interaction between sex and APOE ε4 on cognitive function. However, Ponsford et al. (2011) reported that females APOE ε4 allele carriers over 55 years of age displayed poorer functional recovery at approximately 1 year post injury, as compared to their male counterparts. This result was interpreted by the authors as being related to post-menopausal declines in sex hormones. In contrast, Ost et al. (2008) reported that male APOE ε4 carriers were more likely to have a worse outcome (death or severe disability) than female APOE ε4 carriers after severe TBI. However, given Alexander and colleagues (2007a) found no interaction between sex and APOE status in regards to general functional outcome, it remains possible that no such interaction exists.

To summarise, there is tentative evidence that both age and sex may influence the expression of APOE ε4, with greater age, and being female, both likely to be associated with poorer outcomes in the presence of APOE ε4. However, there has been limited exploration of the effect of age on APOE ε4, and no investigation of the effect of sex on the expression of APOE ε4, in relation to post-TBI cognitive function. It has also been noted that although the APOE ε2 allele is relatively rare, there is evidence that it is dominant over ε3 and ε4 alleles, and may confer some protective effects (Corder et al., 1994; Suri, Heise, Trachtenberg, &

Mackay, 2013). As a result, it is possible that inclusion of APOE ε2 carriers into APOE ε4 carrier and non-carrier groups may obscure any impact of APOE ε4. Although more recent studies have treated APOE ε2 carriers separately (Eramudugolla et al, 2014), this has not routinely occurred, and potentially may also contribute to the mixed findings to date.

The primary aim of the current study was to examine the potential effect of APOE ε4 on the cognitive function of adults with TBI, by longitudinally following recovery at 3, 6 and 12 months following TBI. Here it was specifically hypothesised that carriers of APOE ε4 would have poorer cognitive function than non-carriers. A secondary aim was to examine the relationship between age and sex and APOE status on cognitive recovery from TBI. In line with an antagonistic pleiotropic explanation, it was hypothesised that young adult APOE ε4 carriers (ages 18-50 years) would retain better cognitive function and display faster recovery, than non-carriers of the same age, whereas older (51-70 years) APOEε4 carriers were predicted to experience poorer cognitive function post injury than non-carriers of the same age. In relation to the effect of sex, it was hypothesised that female APOE ε4 carriers would perform worse than non-carriers and male APOE ε4 carriers. Therefore, the aim of this study is to explore the interactions between age and APOE ε4, and sex and APOE ε4, in the context of post-TBI cognitive recovery, without potentially confounding effect of including the APOE ε2 carriers.

Method

Participants

The current study was conducted within an existing longitudinal population study of TBI, the Tasmanian Neurotrauma Register (TNTR), located at the Royal Hobart Hospital in Tasmania, Australia (Australia operates under a no-fault compensation system). Patients

with a diagnosis of TBI, who attended the Royal Hobart Hospital between the years 2003 and 2007 were invited to participate in the TNTR study, and those recruited underwent neuropsychological assessment at time injury, or as soon as possible following remission of post traumatic amnesia (PTA), with follow up assessments at 3, 6 and 12 months following TBI, and then annually up to 5 years post injury. Participants were recruited into the current study during routine follow-up within the larger TNTR study. Institutional ethics approval was obtained for all aspects of this study.

All participants met the following inclusion criteria: They were aged between 18 and 70 years of age, had experienced more than 5 minutes of PTA as estimated by Westmead Scale score (Shores, Marosszeky, Sandanam, & Batchelor, 1986) or by the Galveston Orientation and Amnesia Test (Levin, Odonnell, & Grossman, 1979), and they had no premorbid history of neurological or neurodegenerative disorders, TBI, medicated psychological disorders, or evidence of a developmental delay (full scale IQ (FSIQ) < 70), as estimated by the National Adult Reading Test (NART).

A total of 173 participants agreed to provide DNA samples, however three were unable to be genotyped, resulting in n = 170. Genotype frequencies were determined to be in Hardy-Weinberg equilibrium (χ^2 (5) = 6.050, p = .30). Of these participants, 119 had neuropsychological data available for the time-points of interest. Given the potentially confounding effect of including APOE ε2 carriers in APOE ε4 we intended to examine the influence of the APOE ε2 allele. However, due to insufficient APOE ε2 sample size (n = 12), ε2 carriers were excluded from this study, with the remaining 107 participants comprising two groups: APOE ε4 carrier group (ε4/ε4 and ε4/ε3) and an APOE ε3 homozygote group (ε3/ε3). The demographic and injury related data for the APOE ε4 and APOE ε3 groups is provided in Table 16. As can be seen, there were no significant differences between the groups for age, sex, premorbid IQ, severity of injury, or injury mechanism.

Table 16

Demographic and injury related variables for APOE ε4 carriers (ε4/ε4 and ε4/ε3) and APOE ε3 (ε3/ε3) groups

	APOE ε4 (n = 30)	APOE ε3 (n = 77)	t-test/χ^2	*p* value
Age (years)				
Mean (SD)	40.59 (16.65)	40.19 (16.15)	.115	.909
Sex				
Males	17 (56.67%)	41 (53.25%)	.102	.750
Females	13 (43.33%)	36 (46.75%)		
Estimated FSIQ				
Mean (SD)	103.71 (9.18)	103.50 (9.94)	.101	.920
Post Traumatic Amnesia (PTA)				
Frequency (%age)				
Mild (< 24 hours)	22 (73.33%)	54 (70.13%)	.114	.945
Moderate (< 1 week)	5 (16.67%)	14 (18.18%)		
Severe (> 1 week)	3 (10.00%)	9 (11.69%)		
Glasgow Coma Scale Score (GCS)				
Frequency (%age)				
Mild (13-15)	27 (90.01%)	72 (93.51%)	2.630	.269
Moderate (9-12)	1 (3.33%)	0 (0.00%)		
Severe (3-8)	1 (3.33%)	2 (2.60%)		
Not Recorded	1 (3.33%)	3 (3.89%)		
Injury Mechanism				
Frequency (%age)				
Motor vehicle accident	14 (46.67%)	35 (45.45%)	2.027	.731
Fall	10 (33.33%)	21 (27.27%)		
Assault	4 (13.33%)	9 (11.69%)		
Sports	2 (6.67%)	9 (11.69%)		
Other	0 (0.00%)	3 (3.90%)		

Neuropsychological measures

A battery of seven neurospychological measures was employed: Executive function was assessed by the Trail Making Task form B (TMTB), a measure of cognitive flexibility (Kortte, Horner, & Windham, 2002), and the Controlled Oral Word Association Task (COWAT), a measure of verbal fluency (Lezak, Howieson, & Loring, 2004). Working memory was assessed by the digit span forwards and backwards (DS and DSB), digit span forwards-backwards ratio score (DS-FB), and letter-number sequencing (LNS) subtests of the Wechsler Adult Intelligence Scale IV, and information processing speed was assessed by the information processing (IP Speed) subtest of the Adult Memory and Information Processing Battery, adjusted to control for motor-speed (Coughlan & Hollows, 1985). All tasks have been found to be sensitive to cognitive impairment following mild through to severe TBI (Dikmen, Machamer, Winn, & Temkin, 1995; Kumar, Rao, Chandramouli, & Pillai, 2013; Millis et al., 2001; Ponsford et al., 2000).

Procedure

Participants willing to be included in the current study completed additional informed consent, and provided a buccal swab for DNA collection. Genotyping for APOE was conducted by amplification refractory mutations system (ARMS) PCR, as described by Donohoe, Salomaki, Lehtimaki, Pulkki, and Kairisto (1999). For the current study, neuropsychological data obtained at 3, 6 and 12 month assessments was analysed. Neuropsychological assessors were blind to genotype, and DNA assessors were blind to all participant details.

Statistical analysis

SPSS version 21 was used to conduct all analyses. T-tests and chi-square analyses were used to screen for sex, age, premorbid IQ and severity of injury differences between APOE ε4 carriers and non-carriers. A mixed model approach using full information maximum likelihood (FIML) was conducted to compare the performance of the APOE ε4 and APOE ε3 groups, and to explore the interactions between APOE group and age, and APOE group and sex, for each task at 3, 6 and 12 months post injury. Using the mixed model FIML approach permits a more robust analysis and better accounts for missing data than the traditional general linear model approach (Enders, 2011). An alpha level of $p = .05$ was used to determine significance, and effect size was calculated by estimating *r* values following the formula recommended by Field (2013) for mixed model results.

To explore the relationship between age and APOE status, participants were categorised as either young to middle aged adults (18 to 50 years of age; APOE ε4 n = 23, APOE ε3 n = 51) or older adults (51 to 75 years of age; APOE ε4 n = 7, APOE ε3 n = 26). These age brackets were selected in an attempt to distinguish between participants who were within the reproductive or post-reproductive phase of life, as this is the mean age of menopause for females (Gold, 2011). The interaction between gene (APOE ε4 and APOE ε3) and sex (male and female) was also explored (see Table 16 for group n). While a higher order exploration of the interaction between sex, age and gene would have been valuable, we lacked sufficient sample size to do so.

Results

Tests of normality and skewness indicated that only scores on the TMTB deviated from normal, however as logarithmic and square root transformations did not significantly alter results the untransformed data was analysed. Figures 12 and 13 show the marginal means and standard errors for the age x gene and sex x gene analyses respectively, and the means and standard deviations stratified by genotype are also available in the supplementary material (Tables 17-19 in the supplementary section following chapter discussion).

Age and APOE status

A significant main effect for APOE status (ε4 carriers vs ε3/ε3 genotype) on the TMTB [$F(1, 105.017) = 5.94$, $p = .017$, $r = .23$] was present, with pairwise comparisons revealing that APOE ε4 carriers performed worse than the APOE ε3/ε3 group at 6 months post injury ($p = .012$). A significant interaction between APOE status and time was also revealed for performance on the COWAT [$F(2, 185.67) = 3.94$, $p = .021$, $r = .14$], however subsequent pairwise comparisons found no significant differences at any specific time point.

For TMTB, there was a significant main effect for age [$F(1, 105.02) = 31.86$, $p = .001$, $r = .48$] with the younger age group outperforming the older age group over all time points (all p's = .001), There was also a significant interaction between age and time for the DSFB [$F(2, 198.74) = 3.61$, $p = .029$, $r = .14$], with the younger age group outperforming the older age group at 12 months post injury ($p = .018$). A main effect for age was also revealed on the LNS [$F(1, 105.18) = 4.89$, $p = .029$, $r = .21$] and for IP Speed there was a main effect for age [$F(1, 106.71) = 7.70$, $p = .007$, $r = .26$]. No effects were found for DSB. There was no significant interaction between age and APOE status for any tasks, indicating that older APOE ε4 carriers did not experience worse cognitive outcomes than the APOE ε3/ε3 group following TBI.

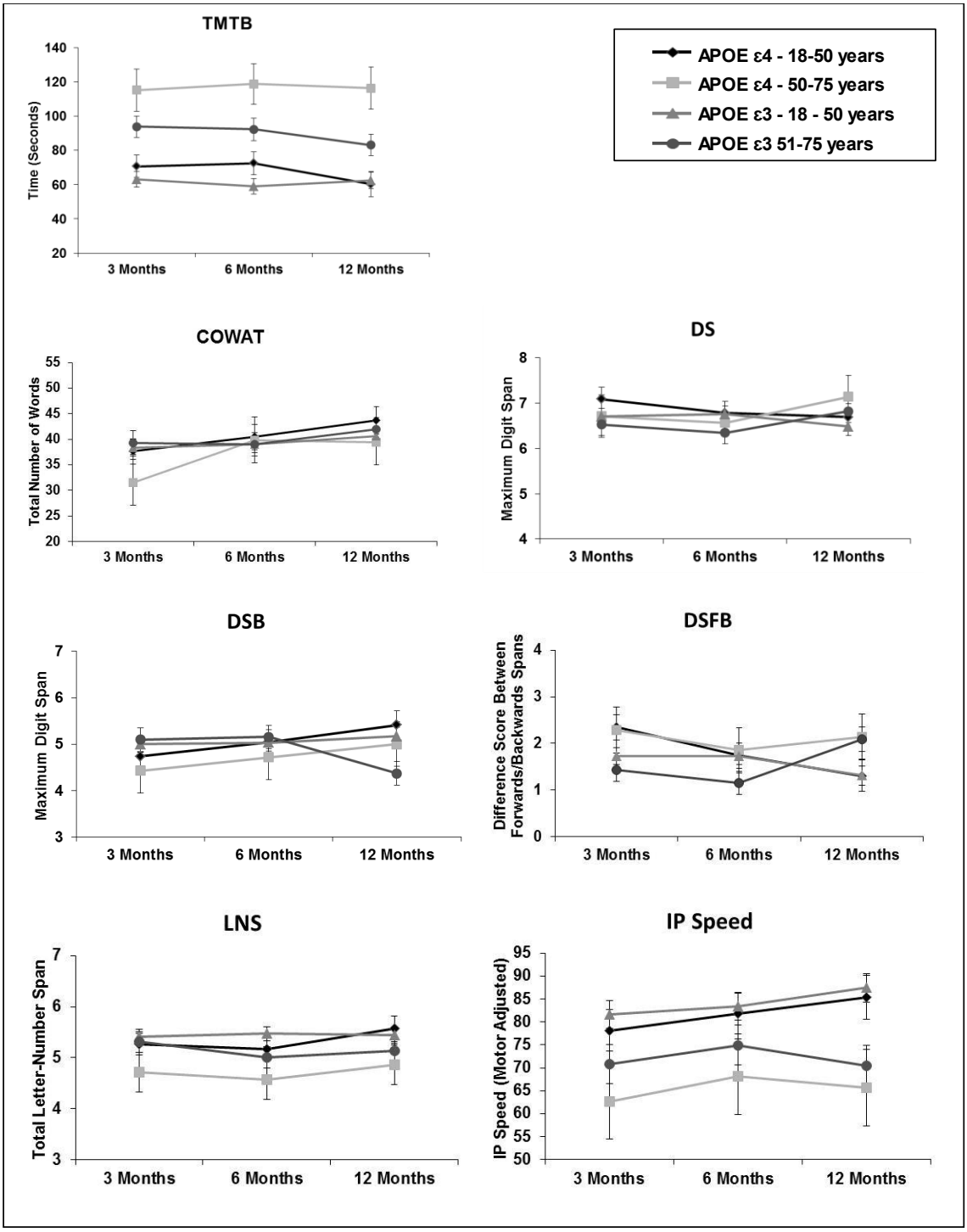


Figure 12. Marginal means and standard errors (SE) for age x APOE group at 3, 6 and 12 months post TBI.

Sex and APOE status

A significant main effect of time on both the COWAT [$F(2, 186.98) = 10.00$, $p = .001$, $r = .23$] and IP Speed [$F(2, 178.54) = 5.62$, $p = .004$, $r = .17$] was present, and there was an interaction between time and APOE status [$F(2, 186.98) = 3.07$, $p = .048$ $r = .13$] however no significant differences were found on subsequent pairwise comparisons. There was also a significant main effect for sex [$F(1, 107.84) = 8.01$, $p = .006$, $r = .26$] with females outperforming males at all time-points. An interaction between sex and APOE status was also revealed [$F(1, 107.84) = 6.02$, $p = .016$, $r = .23$] with pairwise comparisons indicating a trend towards female APOE ε4 carriers outperforming female APOE ε3 homozygotes ($p = .060$). For the DS, there was an interaction between sex and time [$F(2, 194.93) = 3.32$, $p = .038$, $r = .13$] with pairwise comparisons indicating that females outperformed males at 12 months post injury ($p = .033$). A similar interaction was found between sex and time for the LNS task [$F(2, 192.64) = 6.66$, $p = .002$, $r = .18$] again with females outperforming males at 12 months ($p = .046$). There was also a trend for females to outperform males at 6 months ($p = .062$) on this task. No effects were found for TMTB, DSB, or DSFB. Therefore, with the exception of performance on the COWAT, there was no evidence of an interaction between sex and APOE status.

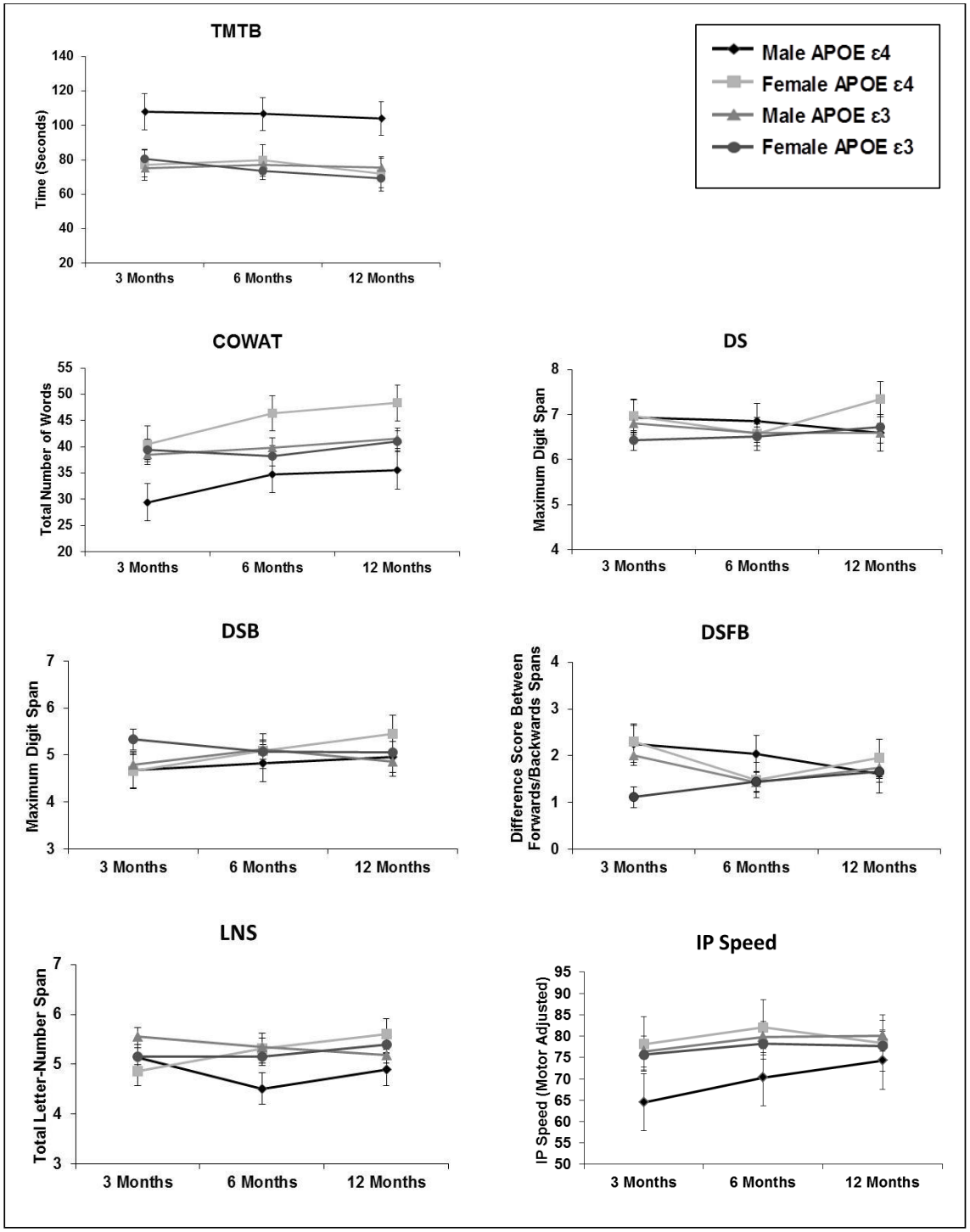


Figure 13. Marginal means and standard errors (SE) for sex x APOE group at 3, 6 and 12 months post TBI.

Discussion

This study sought to explore the direct effect of carriage of an APOE ε4 allele on cognitive function in TBI at 3, 6 and 12 months after injury, and to also determine whether age or sex interact with APOE ε4 in affecting post-TBI cognitive function. While there were no significant differences between APOE ε4 and APOE ε3 groups on measures of information processing speed and working memory, APOE ε4 carriers had poorer performance on the TMTB, a measure of cognitive flexibility, at 6 months post-TBI, and there was evidence of reduced verbal fluency performance on the COWAT. Given that both the TMTB and COWAT are considered to assess aspects of executive function, this finding may indicate that there is a domain specific effect of possessing APOE ε4, whereby executive function is negatively impacted following TBI. Some authors have suggested that APOE ε4 does have a domain specific effect in healthy populations (Rusted et al., 2013; Wisdom, Callahan, & Hawkins, 2011), but no other study of TBI has reported a specific impairment in executive function. In fact, only one other study has reported executive function impairment, and this was in conjunction with impairment to other domains (Ariza et al., 2006). Thus, caution must be applied in interpreting the current findings given the limited supporting evidence for APOE ε4 being associated with a domain-specific effect on executive function.

The second aim was to investigate whether APOE ε4 exerts its effects via an antagonistic pleiotropic mechanism, whereby possession of APOE ε4 confers an advantage in young APOE ε4 carriers but is deleterious in older age. Counter to the expectations from an antagonistic pleiotropic viewpoint, and contrary to the findings of Han and colleagues (2007), we did not find that young adult APOE ε4 performed better than their non-carrier counterparts, nor did we find that older APOE ε4 carriers performed worse than older non-carriers. The contrast with Han et al. is particularly noteworthy given that their sample consisted predominantly of young adult mild to moderate TBI suffers, as did ours. Our

findings also align with the studies by Friedman and colleagues (1999), who found no interaction between APOE status and age on post-TBI cognitive function, and Rapoport et al. (2008), who did find any differences in cognitive recovery in older (> 50 years) TBI participants based on APOE ε4 status.

Interestingly, Evidence from the AD literature suggests that possession of APOE ε4 is associated with increased amyloid accumulation, particularly in late adulthood, and it has been suggested that this increased amyloid pathology is the cause of any APOE ε4 associated cognitive impairment (Harrington et al., 2013; Jack et al., 2015). Furthermore, as mentioned in the introduction, there is evidence that amyloid deposition increases following TBI, and that this production is accelerated in the presence of APOE ε4, even when TBI is considered mild (Hartman et al., 2002; Yang et al., 2015). Should this be the case, it is likely that older TBI sufferers who possess the APOE ε4 allele would have a pre-existing higher amyloid burden, which could then be further exacerbated by the injury. Therefore it is also possible that given the relatively young age of our TBI sample, there was not sufficient amyloid burden to lead to cognitive impairment, despite the presence of APOE ε4. However, the relationship between APOE, amyloid and cognitive function remains uncertain, and it has also been noted that APOE is likely to exert it's effects via a number of neuropathological pathways (Corona & Landreth, 2015; Fitz et al., 2015; Wolf et al., 2013).

There was also no support for our prediction that APOE ε4 females would have poorer performance than non-ε4 females or males; indeed, the only significant interaction between sex and APOE status indicated a trend towards superior performance of APOE ε4 females (as compared to non ε4 females) on the COWAT. While this is counter to previous research in which it was suggested that APOE ε4 females had poorer outcomes than female non-carriers, it must be noted that previous research explored cognitive function in relation to Alzheimer's disease and as such recruited older aged participants (Bartres-Faz et al., 2002;

Beydoun et al., 2012; Mortensen & Hogh, 2001), and that in the only TBI study to note an interaction between APOE status and sex, it was reported that only female APOE ε4 carriers over the age of 55 years experienced poorer outcomes (Ponsford et al., 2011). Thus, it may be that any negative impact of APOE ε4 is reduced in the presence of female sex hormones, but becomes apparent during the post-reproductive life phase when sex hormones are in decline. However, while the interaction was significant, pairwise comparisons only indicated a trend toward APOE ε4 females having better performance than APOE ε3 females, and given that there were no significant interactions between sex and APOE status on any of the other tasks, this effect possibly spurious and must be interpreted with caution.

In addition to being the first study to explore the interaction between sex and APOE status in relation to post-TBI cognition, a further advantage of this study is that it contains one of the largest APOE ε4 samples published to date using neuropsychological tasks to assess recovery in the first 12 months following TBI. Nonetheless, it is likely that larger cohorts may be required to detect any potential genotypic effects, given the reported small to modest effect sizes in this study and other prior studies (Friedman et al., 1999; Ponsford et al., 2011; Teasdale, Murray, & Nicoll, 2005). It should be noted that modest effect sizes are still likely to have clinical significance (Ferguson, 2009), and therefore further investigation is justified. Sample size also precluded us from a more nuanced exploration of the hypotheses. Ideally, analysis of the relationship between age, sex, TBI and APOE would have been useful, as it is likely that age would moderate the effect of sex, especially for females who undergo distinct hormonal changes during menopause (Hoyt & Falconi, 2015). This issue is especially pertinent in the context of APOE, given the association reported between APOE expression and sex hormones, particularly estradiol (Horsburgh, Macrae, & Carswell, 2002; Struble et al., 2007), and therefore we recommend that future studies attempt to explore this relationship.

There is also evidence from the dementia literature that APOE ε4 has a dose-dependent effect, whereby those homozygous for APOE ε4 have greater risk of developing Alzheimer's disease than those heterozygous for APOE ε4 (Corder et al., 1993; Engelborghs et al., 2006). It is also possible that a dose-dependent effect would influence TBI severity, and indeed Ponsford et al. (2007) reported a trend for APOE ε4 homozygotes to have longer PTA and lower GCS scores following injury. Thus, combining APOE ε4 heterozygotes and homozygotes when assessing cognitive function might obscure any APOE ε4 related impairment. Based on the current estimates of the prevalence of the three APOE genotypes, it can be estimated that approximately 3% of the population is likely to have the ε4/ε4 genotype, and consistent with this estimation, three of our APOE ε4 carriers were homozygous for ε4 (2.5% of total sample – see supplementary material). Unless large, multi-site investigations are conducted, or results are reported by genotype, thereby permitting meta-analytic approaches to be applied, it will prove difficult to obtain sufficient sample sizes to separate APOE ε4 homozygotes and heterozygotes. Nevertheless, exploring dose-dependent effects in terms of post-TBI cognitive function may prove enlightening.

Alternatively, APOEε4 may have region-specific effects, which could also explain the discrepancy between Alzheimer's disease and TBI findings. In TBI, frontal regions are frequently the most damaged (Levine et al., 2006) whereas the neurodegeneration associated with Alzheimer's disease typically commences in the subcortical and temporal regions, before progressing to frontal and parietal areas (Delacourte et al., 1999; Double et al., 1996). Indeed, emerging evidence from Alzheimer's disease research suggests there is a region specific effect of APOE ε4, and that temporal areas may be more negatively impacted by APOE ε4 possession (Hostage, Choudhury, Doraiswamy & Petrella, 2014; Westlye et al., 2012). Therefore, any deleterious effects of APOE ε4 may be diminished when cognitive impairment is primarily a consequence of frontal lobe damage, rather than sub-cortical and/or

temporal damage. However, given the often widespread damage associated with TBI, and the limited empirical support to date, this possibility remains putative, and requires further exploration.

There are a number of limitations to the present study. Firstly, participants were mainly identified as having sustained a mild to moderate TBI, and there is tentative evidence that any detrimental effect of APOE ε4 may only be apparent when injury is severe (Ariza et al., 2006; Millar, Nicoll, Thornhill, Murray, & Teasdale, 2003; Noé et al., 2010). The distribution of TBI severity in the present sample, however, is consistent with the prevalence rates in the general population in Australia and from other Western countries (Fortune & Wen, 1999; Tagliaferri, Compagnone, Korsic, Servadei, & Kraus, 2006). As such, the present results provides ecological evidence that APOE status is unlikely to be a useful biomarker in establishing who might experience poorer cognitive outcomes post-injury, at least for the majority of individuals with TBI. Nonetheless, exploration of the impact of APOE ε4 on post-TBI cognitive function (including the potential interactions between APOE and sex or age) in moderate to severe TBI cohorts may prove fruitful.

Given that the antagonistic pleiotropic mechanism is associated with reproductive life-phases, we chose to categorise old age as over 50 years, based on the average age of menopause for females (Gold, 2011). However, we acknowledge that the distinction between the reproductive and post-reproductive life-phase is less distinct for males, and furthermore, we were unable to confirm the menopausal status of our female participants. Although one of the strengths of the current study was categorising participants into young and old age groups, it must be noted that the majority of participants fell into the younger age category (18-50 years n = 74, 51-70 years n = 33). Irrespective of APOE status, it has been established that older age is associated with poorer outcomes following TBI (Ponsford, 2013; Senathi-Raja, Ponsford, & Schonberger, 2010), and therefore it is possible that the lack of effects

reported here is due to the relatively young age of our sample. Similarly, as would be expected given that males are more likely to sustain TBIs (Fortune & Wen, 1999), we had fewer females than males in our sample. Therefore, although we used statistical methods that were robust to unequal sample sizes, a larger number of participants over 50 years of age and more female participants would have permitted a more unbiased investigation.

This study did not examine the effect of APOE on learning and memory. This was due to this study being part of a larger, already established population study, with pre-existing assessment protocols. While the tasks used in the present study are sensitive to change following TBI, and covered a range of domains, there is some evidence that verbal memory is more frequently impaired in APOE ε4 carriers than other domains (Anderson et al., 2009; Crawford et al., 2002; Lawrence, Comper, Hutchison, & Sharma, 2015). Therefore, a future examination of APOE and learning and memory in a TBI sample is warranted.

In conclusion, our results suggest that APOE ε4 is not associated with impaired cognitive function following TBI, and therefore APOE genotype may not have prognostic value in this context. Moreover, we did not find any evidence that age interacts with APOE ε4, and as such the hypothesis that APOE may function via an antagonistic pleiotropic mechanism was not supported. We also did not find a systematic interaction between sex and APOE ε4, despite the reported effect of sex hormones on the expression of APOE. In the absence of supporting evidence regarding age and sex interactions with APOE, our findings are tentative and must also be interpreted in light of the fact that many of our sample sustained mild TBI, and the majority were relatively young adults. Larger studies, particularly in individuals with moderate to severe TBI, are needed to more thoroughly examine and enhance our understanding of the relationship between age, sex and APOE status.

Supplementary Material

Table 17

Means and standard deviations for neuropsychological test performance by genotype at 3 months

	APOE genotype					
	ε4ε4 (*n* = 3)	ε4ε3 (*n* = 27)	ε4ε3 (n = 4)	ε3ε3 (*n* = 77)	ε3ε2 (*n* = 10)	ε2ε2 (*n* = 2)
TMTB	75.64 (32.92)	79.52 (36.45)	71.67 (18.58)	72.67 (29.29)	94.10 (46.97)	62.50 (2.12)
COWAT	38.12 (11.65)	38.38 (11.86)	27.33 (7.37)	38.58 (11.40)	40.30 (10.81)	35.00 (15.56)
DS	6.69 (1.18)	7.04 (1.02)	7.67 (2.31)	6.66 (1.21)	6.30 (1.25)	5.00 1.14)
DSB	4.86 (1.21)	4.67 (1.21)	5.33 (1.53)	5.04 (1.24)	4.30 (.67)	3.50 (.71)
DSFB	1.91 (1.39)	2.37 (.97)	2.33 (1.53)	1.63 (1.32)	2.80 (2.35)	1.50 (.71)
LNS	5.28 (.95)	5.11 (.89)	6.33 (1.53)	5.38 (1.00)	5.11 (.78)	4.50 (.71)
IP Speed	76.25 (22.24)	76.59 (23.34)	73.00 (7.94)	78.17 (21.00)	69.67 (28.97)	63.00 (1.41)

Table 18

Means and standard deviations for neuropsychological test performance by genotype at 6 months

	APOE genotype					
	ε4ε4 (*n* = 3)	ε4ε3 (*n* = 27)	ε4ε3 (n = 4)	ε3ε3 (*n* = 77)	ε3ε2 (*n* = 10)	ε2ε2 (*n* = 2)
TMTB	152.00 (107.68)	76.60 (42.71)	64.75 (30.41)	68.74 (31.46)	66.40 (27.25)	95.50 (40.31)
COWAT	21.33 (7.02)	42.41 (11.94)	31.00 (8.83)	39.05 (11.50)	42.20 (10.44)	44.00 (.00)
DS	7.00 (.00)	6.70 (1.53)	7.25 (2.06)	6.63 (1.28)	6.40 (1.51)	5.00 (.00)
DSB	5.67 (2.08)	4.89 (1.09)	5.50 (2.38)	5.08 (1.38)	5.10 (.74)	4.00 (1.41)
DSFB	1.33 (2.08)	1.81 (1.30)	1.75 (.96)	1.54 (1.31)	1.30 (1.06)	1.00 (1.41)
LNS	4.67 (1.15)	5.08 (1.20)	6.25 (2.06)	5.32 (1.11)	5.00 (1.16)	4.00 (1.41)
IP Speed	63.00 (10.54)	80.38 (27.14)	86.25 (19.31)	81.11 (20.92)	76.10 (24.17)	68.00 (8.49)

Table 19

Means and standard deviations for neuropsychological test performance by genotype at 12 months

	APOE genotype					
	ε4ε4 (*n* = 3)	ε4ε3 (*n* = 27)	ε4ε3 (n = 4)	ε3ε3 (*n* = 77)	ε3ε2 (*n* = 10)	ε2ε2 (*n* = 2)
TMTB	159.00 (107.48)	68.68 (27.72)	54.50 (6.36)	68.41 (27.45)	77.80 (43.16)	85.50 (3.54)
COWAT	25.00 (1.41)	45.85 (12.81)	33.50 (14.85)	40.83 (12.08)	46.20 (11.30)	49.00 (15.55)
DS	6.50 (.71)	6.75 (1.55)	7.50 (2.12)	6.65 (1.42)	6.80 (1.14)	4.50 (.71)
DSB	4.50 (.71)	5.20 (1.64)	6.00 (2.83)	5.03 (1.17)	5.30 (.95)	3.50 (.71)
DSFB	2.00 (1.41)	1.55 (1.82)	1.50 (.71)	1.61 (1.30)	1.50 (1.27)	1.00 (1.41)
LNS	4.00 (.00)	5.40 (.99)	6.50 (2.12)	5.35 (1.07)	5.30 (.68)	5.00 (.00)
IP Speed	58.00 (.00)	84.33 (24.15)	102.50 (13.44)	79.78 (22.99)	74.00 (27.22)	76.00 (4.24)

Chapter 7: General Discussion

Overview of Thesis Aims and Outcomes

The primary aim of this thesis was to explore the role of the APOE gene in post-TBI cognitive function during the first 12 months following injury, with a focus on the impact of the APOE ε4 allele. A series of related research questions were also addressed, with the intention of providing a more nuanced and rigorous investigation of the role of the APOE gene in relation to cognitive function following TBI. These included the aim of investigating the interaction between age and APOE ε4, given the tentative evidence that APOE may operate via an antagonistic pleiotropic mechanism; and examining the relationship between sex and APOE, given that the expression of APOE is known to be influenced by sex hormones, and there is some evidence that APOE ε4 females may be more vulnerable to any detrimental impact of this allele. Moreover, given the proposition that APOE ε2 might be both ameliorative and dominant over APOE ε4 and ε3, it was intended that by either excluding APOE ε2 carriers from the APOE ε4 carrier and non-carriers groups, or treating them as a separate group, a clearer understanding of the impact of APOE ε4 would be obtained. It was also hoped that it would be possible to explore of the effect of APOE ε4 when injury is moderate to severe. Alongside these research objectives, a methodological study was undertaken to consider the impact of missing data in clinical assessment, by modelling the effect of missingness using a subset of the current thesis' dataset. Collectively, these research objectives were executed through a series of four studies. The following sections summarise the aims and outcomes of each of these studies.

Chapter 2 – Discussion and Key Findings

Despite the growing number of publications describing the impact of APOE ε4 on cognitive function following TBI, at the time of writing there was yet to be a meta-analytic

investigation of the extant literature. The aim of this study was to provide such an analysis, focusing on investigations that used psychometrically and clinically validated cognitive tasks in the initial 12 months following TBI.

The meta-analyses revealed that there were no significant differences between APOE ε4 carriers and non-carriers in terms of general cognitive function, or in any of the specific domains that were assessed. It was noted that there is tentative evidence that any detrimental effects of the APOE ε4 allele may only be apparent in more severe injury (Mannix et al, 2013; Millar et al., 2003), and that age may moderate the expression of APOE in clinical populations (Chang et al., 2011; Han & Bondi, 2008), but that these factors were yet to be explored in relation to post TBI cognitive function. Limitations of the meta-analysis and the related literature were also discussed, including inconsistent use of injury severity measures and assessment tasks, which hindered the ability to compare outcomes, and the routine categorising of APOE status as either APOE ε4 carriers or non-carriers. Dichotomising APOE in this manner means that the potentially ameliorative effect of APOE ε2 is not considered separately, and inclusion of APOE ε2 carriers in the APOE ε4 and non-ε4 groups may confound findings related to the impact of APOE ε4. The importance of consistent reporting of injury and demographic factors, stratified by APOE status, and issues regarding replication and publication bias in the context of candidate gene x environment studies more broadly, were also highlighted. These findings were used to direct the subsequent studies in this thesis, with the intention of providing a more nuanced and robust investigation of the relationship between APOE genotype and post-TBI cognitive function.

Chapter 4 – Discussion and Key Findings

The aim of chapter 4 was to explore the relative efficacy of deletion, single, and multiple imputation techniques. This was undertaken by randomly selecting a complete

subsample of the current thesis' data, calculating the true parameters, then deleting a subset of the data to create a simulated dataset in which values were missing at random. Estimated parameters were then obtained for this hypothetical data by applying deletion, mean and regression substitution (single imputation) and multiple imputation approaches and the resulting parameter estimates were compared to the known true parameters of the sample. This technique revealed that the multiple imputation approach provided more accurate estimates that the deletion or single imputation approaches, and was less likely to underestimate the true variance.

The finding that multiple imputation was better able to estimate parameters, and that traditional approaches may reduce accuracy, highlights the need to consider missing data when conducting analysis. Currently, multiple imputation cannot easily be used in some contexts, including longitudinal research, however it is expected that future statistical programmes will allow multiple imputation to be more easily applied, and therefore researchers should be aware of the benefits of using more sophisticated approaches to compensate for missing data, and aim to use them where practical.

Chapter 5 – Discussion and Key Findings

The primary aim of this study was to determine whether APOE ε4 significantly contributed to cognitive outcome during the early recovery period following TBI, once other injury and demographic variables were accounted for. Regression analyses indicated that once injury severity, age, and premorbid IQ were accounted for, there was no evidence that APOE status (APOE ε4 homozygotes and heterozygotes versus APOE ε3 homozygotes) contributed to cognitive outcome during the early recovery phase. The ancillary analyses also indicated that possessing APOE ε2 was not associated with better cognitive function, and that APOE ε4 carriers who sustained a moderate to severe TBI did not have poorer outcomes

than the APOE ε3 group. It must be noted that these ancillary analyses relied on small samples, and the findings should be interpreted with caution. However, considered collectively, the findings from this study suggest that APOE status is unlikely to influence cognitive function (specifically processing speed, executive function and working memory) during the initial recovery period following TBI, regardless of injury severity. It could also be argued that this study provided evidence that incorporating APOE ε2 carriers into APOE ε4 and non-ε4 groups may not impact meaningfully on results, however given the samples size, this finding should be viewed with caution until replicated.

Chapter 6 – Discussion and Key Findings

The final study in this thesis explored the effect of APOE ε4 at 3, 6, and 12 months following TBI. As well as investigating the direct effect of APOE ε4, the aim of this study was to also determine whether age or sex interacted with APOE ε4. A particular interest was whether or not APOE ε4 was expressed via an antagonistic pleiotropic mechanism, whereby possession of the ε4 allele conferred beneficial effects during the reproductive life phase and detrimental effects in the post-reproductive life phase. While there was some evidence that APOE ε4 carriers had poorer performance than non-carriers on the TMTB and COWAT, there were no significant differences detected across the majority of tasks. Further, there was no evidence of an interaction between age and APOE, and on only one task was an interaction between sex and APOE, in which, contrary to the hypothesis, APOE ε4 females performed better than both male and female APOE ε3 homozygotes and APOE ε4 males. In accordance with the results in chapter 5, this study indicated that APOE ε4 is not associated with poorer cognitive function after TBI, and that age and sex do not appear to influence the expression of the ε4 allele, although given the main effect of APOE ε4 on the TMTB and

COWAT – both measures of executive function - it is possible that there is a domain-specific effect of APOE ε4 on executive function.

General Discussion

This thesis raises questions regarding the impact and effect of APOE polymorphisms on post-TBI cognitive function. The meta-analyses reported in chapter 2 found no evidence of a detrimental effect of APOE ε4, for either general cognitive function, or within the specific domains of executive function, working memory, verbal or visual memory. The data obtained for these meta-analyses was from studies that had investigated the impact of APOE ε4 on cognitive function in the first 12 months following TBI, using psychometrically validated tasks. It must be noted that publication bias and lack of replication is argued to be particularly prevalent in genetic investigations relating to neuropsychological and neuropsychiatric function (Dick et al., 2015; Duncan & Keller, 2011). Should such a publication bias be true across the APOE/TBI literature, this would in fact bolster the null effect reported here. Although susceptible to the effects of publications bias, meta-analyses have been reported to provide a more accurate estimation of effect sizes than individual studies even when only a small number of studies are included (Cumming, 2012), and therefore the findings from this analysis are compelling.

Similarly, the finding reported in chapter 5 revealed no evidence of a detrimental effect of APOE ε4 during the initial recovery period when other injury and demographic variables were accounted for. This lack of effect remained even when only participants with moderate to severe injury were assessed. The longitudinal study reported in chapter 6 also indicated that APOE ε4 was not associated with reduced or delayed recovery of cognitive function at 3, 6 or 12 months post-injury, with the exception of poorer performance by APOE

ε4 carriers on the TMTB at 6 months, and some evidence of reduced performance by APOE ε4 carriers on the COWAT, although subsequent pairwise comparisons did not reveal differences at any given time-point. As both tasks are considered to measure aspects of executive function (Bagiella et al., 2010; Kortte et al, 2002; Wilde et al; 2010), this finding may indicate a domain-specific effect of APOE ε4, but in the absence of supporting evidence from previous studies, this hypothesis remains speculative.

Also of interest in this thesis was the proposition that age and/or sex may interact with APOE ε4. In relation to age, it was hypothesised that the APOE gene may operate via an antagonistic pleiotropic mechanism, whereby possession of the APOE ε4 allele might confer some benefits prior to and during the reproductive life phase, but become deleterious in post-reproductive life. This prediction was instigated by the diverse range of findings outside of the TBI literature which indicated that APOE might function via this process (Chang et al., 2011; Jasienska, Ellison, Galbarczyk, Jasienski, & Kalemba-Drozdz, 2015; Jochemsen, Muller, van der Graaf, & Geerlings, 2012; Kulminski et al., 2011; Rusted et al., 2013). Contrary to expectations, there was no evidence of an interaction between age and APOE status in relation to post-TBI cognitive function.

The prediction that there may be an interaction between APOE status and sex was based on evidence from cellular and animal studies which indicated that oestrogen increases levels apoE (Struble, Nathan, Cady, Cheng, & McAsey, 2007), but is less effective for APOE ε4 (Lambert et al., 2004; Nathan, Barsukova, Shen, McAsey, & Struble, 2004). Despite the evidence from related avenues of research, there has been little exploration of the interaction between age and APOE status in terms of post-TBI cognition, and no studies exploring the possibility that sex may interact with APOE status in relation to post-TBI cognition. Thus it was proposed that there would be an interaction between sex and APOE, whereby APOE ε4 females would have poorer outcomes than APOE ε3 females, or males of either APOE status.

These hypotheses were explored in the longitudinal study described in chapter 6. One interaction between APOE status and sex was found on the COWAT, however pairwise comparisons revealed this was a non-significant trend in the opposite direction to that hypothesised, whereby APOE ε4 females had slightly better performance than the APOE ε3 females. Given the lack of theoretical support, and the lack of other significant differences, it is likely that this effect was spurious.

With the exception of tentative evidence suggesting that executive function might be detrimentally impacted by presence of the APOE ε4 allele, the findings from this thesis indicate that APOE ε4 is not associated with poorer cognitive function following TBI, even when demographic and injury related factors which have been reported to moderate outcomes are incorporated into analysis. This supports the meta-analytic study that was initially undertaken. Therefore, it appears unlikely that determining an individual's APOE genotype will have any prognostic value in relation to estimating post-TBI cognitive outcomes.

This thesis has a number of strengths. Firstly, incorporating both meta-analytic and original, longitudinal research permitted a broad, multifaceted investigation, with the findings from each study converging to indicate little or no effect of APOE ε4. The inclusion of age and sex as interacting factors was also beneficial given that, despite evidence from animal TBI models, the possible interaction between APOE status and sex or age had not been systematically explored in adult TBI populations. The treatment of APOE ε2 carriers as a separate group also allowed a more focused comparison of the three APOE alleles, which has not typically occurred in previous literature. Finally, at the time of writing, the studies in chapters 5 and 6 contained some of the largest published samples of TBI participants in which both APOE genotyping and cognitive assessment was undertaken within 12 months of injury. Moreover, a range of neuropsychological tasks that are known to be sensitive to change following TBI were employed. Overall, these factors have allowed a more focused

and integrative exploration of the relationship between APOE and cognitive function following TBI than has occurred to date.

Issues Relating to Assessment of Injury and Cognitive Function in TBI Populations

TBI severity: Sample characteristics and comparability of GCS and PTA estimates.

It has been suggested that any detrimental effect of APOE ε4 may only be apparent when injury is severe (Ariza et al., 2006; Millar, et al., 2003; Noe, et al., 2010), and although a preliminary analysis was conducted in this thesis on only moderate to severe participants at the acute recovery phase, the majority of participants recruited for this thesis were classified as having sustained mild TBIs. Arguably, this thesis reflects typical prevalence rates of mild TBI in western populations (Fortune & Wen, 1999; Tagliaferri, Compagnone, Korsic, Servadei, & Kraus, 2006), and therefore provides an ecologically valid investigation. As such the lack of differences between APOE ε4 and non-carriers reported here suggests that this biomarker is unlikely to be useful for predicting post-injury cognitive function for the general TBI population. However, the possibility remains that APOE status is important when injury is moderate or severe, and therefore further investigation is needed in this population.

There are also discrepancies in estimation of injury severity depending on the scale used, and this issue has also been identified in previous research (Sherer et al., 2008). In the studies reported in chapters 5 and 6, PTA estimates classified more participants as having moderate-severe injury severity than GCS. GCS is often used as an estimator of injury severity, however there is compelling evidence that PTA is more closely associated with outcome, and as such may be a better estimate of injury severity (Balestreri et al., 2004;

Perrin et al., 2015). Although PTA and GCS are both provided here to improve ability to compare this study to other studies, this research highlighted the disparity that can arise from assessing injury severity using GCS and PTA. There are two key concerns regarding injury estimations in relation to this thesis: Firstly, the studies included in the meta-analysis (chapter 2) utilised a range of approaches to estimate severity, making comparisons challenging. Secondly, as mentioned above, in the current sample the use of GCS resulted in fewer participants being identified as having moderate or severe TBI than when PTA estimates were used. This issue is unsurprising given the reported disparity between these measures (Sherer et al., 2015; Sherer et al., 2008), and is unavoidable until more accurate biomarkers of severity are determined. Although it is recognised that PTA estimates tend to better predict outcome, GCS remains the more common measure, partly due to its use in the emergency medical setting, which makes it an easily obtainable estimate, but the differences reported here emphasise the need for researchers to be aware of the discrepancies and reduced comparability between studies using GCS or PTA. It is therefore suggested that reporting both GCS and PTA in future studies will allow more accurate comparisons between studies, and will also be especially useful should meta-analytic or other integrative approaches be employed in future.

Scope of cognitive assessment.

The participants recruited for the studies in chapters 5 and 6 were part of a larger population study, with the neuropsychological testing protocols having already been established. As a result only processing speed, executive function and working memory tasks were used to assess neuropsychological function. In the broader TBI literature, attention, executive function and memory are the domains most commonly impaired, even in mild TBI (Mathias & Wheaton, 2007; McDonald, Flashman & Saykin, 2002; Rabinowitz &

Levin, 2014; Rohling et al., 2011). Although the meta-analysis in chapter 2 did not find that either verbal or visual memory were impacted by APOE status, there is some evidence from previous findings that APOE ε4 may influence memory, and particularly verbal memory, more than other cognitive domains (Anderson et al., 2009; Ariza et al., 2006; Crawford et al., 2002; Eramudugolla et al., 2014; Noe, Ferri, Colomer, Moliner, & Chirivella, 2010; Sundström et al., 2004). Therefore including measures of visual and verbal memory would have broadened the scope of this thesis.

A further issue identified in this thesis is the inconsistent use of cognitive tasks to assess outcome. A broad range of tasks have been utilised in TBI research (Tate et al., 2013), which can make comparisons between studies challenging, and thus had implications for the meta-analysis reported in chapter 2. Clearly a range of tasks are needed to adequately cover the range of cognitive functions, however it may be useful for future assessment to use some core tasks (with the inclusion of additional measures where needed) to allow more accurate comparison between studies, and to permit later integrative analyses such as meta-analysis to be undertaken. Wilde and colleagues (2010) and Bagiella et al. (2010) have both recommended a range of tasks that they suggest should be routinely incorporated into cognitive assessment after TBI. As outlined in chapter 2 of this thesis, Wilde et al. also provide a stratified protocol which encourages the inclusion not only of validated tasks that are known to be sensitive to change following TBI but also of emerging measures, which will hopefully lead to the development and validation of new methods of assessment. As well as providing data from measures that are known to be reliable and valid, this may also encourage the evolution of neuropsychological assessment in relation to TBI. It is therefore recommended that researchers consider utilising the tasks recommended by Wilde et al to ensure a comprehensive coverage of all cognitive domains, and to foster the development of new assessment tasks.

It should also be noted that premorbid intellectual capacity was estimated by using the NART, rather than education level. It has been reported that the NART may underestimate premorbid functioning when TBI is more severe, however this effect appears to diminish when TBI is mild or moderate (Morris et al., 2005). There is also some evidence from non-impaired populations indicating that the NART may have reduced reliability as a predictor for cognitive measures other than verbal intelligence and FSIQ (Schretlen, Buffington, Meyer, & Pearlson, 2005). While the majority of the participants in the current thesis had sustained mild to moderate injuries, it is possible that inclusion of education level may have improved accuracy in estimating premorbid function, and future studies may benefit from using both reading and education tasks concurrently.

Issues Relating to APOE; ε4 dose-dependency, and gene-gene interactions

It is possible that APOE ε4 is expressed in a dose-dependent manner, whereby any detrimental effects would be more pronounced when the ε4 allele is present in the homozygous condition. There is evidence from non-TBI literature that there is a dose-dependent effect for APOE ε4 in relation to increased risk for APOE ε4 homozygotes developing AD (Corder et al., 1993), and evidence that APOE ε4 homozygotes experience greater age-related cognitive decline than APOE ε4 heterozygotes (Kang, Logroscino, De Vivo, Hunter, & Grodstein, 2005; Nilsson et al., 2006). In TBI literature, researchers have consistently grouped ε4 heterozygotes and homozygotes together, which is pragmatic given the small number of individuals who are likely to be homozygous for ε4, but unfortunately this means that there is scant evidence to determine whether there is a dose-dependent effect for post-TBI cognitive function. The exception to this is a study by Ponsford and colleagues (2007) in which a trend was observed for those with the ε4/ε4 genotype to have poorer general outcome following TBI, however the researchers were unable to explore this due to

insufficient sample size. Approximately 3% of the population is likely to have the ε4/ε4 genotype, and so exploring the dose-dependency effect of APOE ε4 will prove challenging unless a multi-centre or meta-analytic approach is taken, and given the prevalence of this genotype the clinical relevance may be limited. Nonetheless, it may be worth exploring this possibility in order to better understand the role of APOE in relation to cognitive function. Although this thesis also groups APOE ε4 homozygotes and heterozygotes together, the descriptive data for each genotype has been provided as part of chapter 6, in the hopes that this may be utilised by future meta-analytic research, and it is suggested that future research provide similar data.

There have also been calls for a more integrative approach when exploring the role of candidate genes in relation to psychological function and illness, and in particular the need to investigate gene x gene interactions has been stressed (Dick et al., 2015). It may therefore prove fruitful to explore whether the APOE gene interacts with other genes in the context of post-TBI cognition. Indeed, there is evidence from other clinical groups that the brain-derived neurotrophic factor (BDNF) gene may interact with APOE in relation to memory function (Richter-Schmidinger et al., 2011; Ward et al., 2014), and it has been reported that allelic variation BDNF and also in other genes such as catechol-O-methyltransferase (COMT), Ankyrin repeat and kinase domain-containing 1 (ANKK 1), and kidney and brain expressed protein (KIBRA) could influence cognitive outcome following TBI (Lipsky et al., 2005; McAllister et al., 2012; Wagner et al., 2012; Yue et al., 2015). Thus, exploring polygenetic effects in relation to post-TBI cognitive function is warranted, and with the growing availability of sophisticated and time- and cost-efficient genotyping, this avenue of research is becoming increasingly feasible.

Limitations of treating age and sex independently

Although sex and age differences have been discussed separately here, it is important to note that both factors should be considered conjointly. Given that the antagonistic pleiotropy hypothesis suggests that declines would not be seen until the post-reproductive life phase, categorising participant age by considering reproductive status, rather than treating age as a linear variable, may improve the ability to detect any age-related changes. This is particularly apposite for females, who typically experience a distinct reduction in sex hormones during menopause, whereas males have a more gradual and linear decline in sex hormone levels (Hoyt & Falconi, 2015). Nevertheless, both males and females may experience cognitive declines that are a function of changes in sex hormones across the lifespan which may in turn interact with APOE genotype (Holland, Bandelow, & Hogervorst, 2011; Yaffe, Haan, Byers, Tangen, & Kuller, 2000). Unfortunately sample size precluded an exploration of this interaction, but future research into the effect of APOE ε4 may benefit from considering age and sex differences in an integrated manner, ideally either through using mixed modelling or moderation analysis rather than employing traditional linear approaches, which are unlikely to detect non-linear relationships and outcomes.

Furthermore, it was not possible to categorise females by menopausal status in the current thesis. If this had occurred, it may have been possible to conduct additional analyses involving only pre-menopausal females.

Directions for Future Research

A region and/or domain-specific effect for APOE ε4?

Imaging studies indicate that APOE ε4 is associated with greater atrophy of hippocampal and entorhinal regions in both healthy and clinical samples (Heise, Filippini,

Ebmeier, & Mackay, 2011; Hostage, Choudhury, Doraiswamy, & Petrella, 2014; O'Dwyer et al., 2012; Westlye et al., 2012), and that presence of the APOE ε4 allele accelerates atrophy in hippocampal and temporal regions in the context of AD (Manning et al., 2014), although it must be noted that this was not observed a TBI study (Isoniemi, Kurki, Tenovuo, Kairisto, & Portin, 2006). Given that the neurodegeneration associated with AD typically commences in the subcortical and temporal regions, before progressing to frontal and parietal areas (Delacourte et al., 1999; Double et al., 1996), whereas TBI is associated with more diffuse and variable damage, and is most commonly associated with frontal-lobe dysfunction (Bendlin et al., 2008; Levine et al., 2006; MacKenzie et al., 2002), it is possible that the effects of the APOEε4 allele may be diminished when structural atrophy is diffuse or not focussed in temporal regions.

Such an effect would also explain the evidence that possession of the APOE ε4 allele might be specifically associated with poorer verbal and episodic memory, as has been reported in TBI and non-clinical populations (Anderson et al., 2006; Ariza et al., 2006; Crawford et al., 2002; Lawrence et al, 2015; Wilson et al., 2002). This thesis did not use measures of verbal or episodic memory, however these findings suggest that cognitive functions such as memory, which rely heavily on hippocampal activation, may be more vulnerable to the effects of APOE ε4 than other domains following TBI, and highlights the need to use sensitive and specific cognitive assessments, rather than rely on general outcome measures to estimate cognitive impairment. It was not possible to test verbal memory in the current thesis, but the use of specific tasks in this thesis also indicated the possibility that APOE ε4 may have domain specific effects insofar as there was tentative evidence that executive function was negatively impacted by the presence of the APOE ε4 allele, although this finding does not align with the evidence of preferential atrophy of temporal/hippocampal regions.

Premorbid effect versus TBI-induced impairment

There is also evidence that healthy APOE ε4 carriers demonstrate poorer cognitive performance and reduced white-matter integrity. In line with the proposition that APOE ε4 may be associated with region-specific effects, hippocampal and temporal regions appear to have reduced myelination in healthy APOE ε4 carriers, with evidence that this is associated with specific reductions in memory (Heise et al., 2011; Honea, Vidoni, Harsha, & Burns, 2009; Wilson et al., 2002). As discussed in chapter 2, a recent meta-analysis by Wisdom and colleagues (2011) also indicated that APOE ε4 was associated with modest decreases in episodic memory in healthy populations. As well as providing further support for a region-specific effect, these findings may indicate that any reduction in the cognitive function of APOE ε4 TBI sufferers is reflective of premorbid differences, and that APOE ε4 carriers do not experience greater impairment as a direct result of injury. However, in the only published study to date that has compared pre- and post-injury cognition, Sundström and colleagues (2004) reported that when compared to pre-injury test performance, APOE ε4 carriers demonstrated post-injury cognitive declines in attention and memory, whereas non-carriers did not. Therefore, it is feasible that possession of APOE ε4 does increase severity of cognitive impairment after TBI, but until further research occurs which uses either a within groups approach or includes a healthy age-matched control group, this supposition remains speculative.

Does APOE ε4 influence cognition by increasing amyloid burden?

It has been established that that accumulation of amyloid increases with both age (Jack et al., 2015), and following TBI (Chen et al., 2004; Smith et al., 2003; Yang et al., 2015), and it appears there is a relationship between APOE and amyloid pathology, with Shinohara, Petersen, Dickson, and Bu (2013) reporting an inverse correlation between accumulation of amyloid pathology and level of apoE, with others finding that amyloid

accumulation increases in the presence APOE ε4 (Hartman et al., 2002; Nicoll et al., 1995; Yin et al, 2014). As discussed in the introduction and in chapter 6, this could explain the connection between APOE ε4 and increased risk of AD (Bales et al., 2009), and cognitive impairment (Harrington et al., 2013; Jansen et al, 2015; Nilsson et al., 2002), and therefore the relationship between APOE, amyloid deposition and post-TBI cognitive function warrants exploration. Specifically, it is possible that when APOE ε4 carriers sustain a moderate to severe TBI in later life, the existing amyloid burden results in poorer outcomes. This hypothesis would also align with a region-specific explanation of the effect of APOE ε4, given that amyloid aggregation is known to commence in medial-temporal regions (Thal, Rub, Orantes, & Braak, 2002). However, there is some evidence that presence of amyloid pathology is not associated with poorer cognitive function in TBI (Kawai et al., 2013), and it must also be noted that the relationship between APOE and amyloid accumulation is likely to be complex, and that APOE ε4 is also likely to impact on cognition via a number of neuropathological pathways beyond amyloid accumulation (Corona & Landreth, 2015; Fitz et al., 2015; Wolf et al., 2013). Nonetheless, the relationship between APOE genotype and amyloid deposition and clearance could potentially account for the antagonistic pleiotropic mechanism that has been proposed by some researchers.

Conclusions

The aim of this thesis was to explore the role of APOE ε4 in cognitive function after TBI. Collectively, the meta-analytic and original research undertaken for this thesis indicted that possession of APOE ε4 is unlikely to be associated with poorer neuropsychological function in the first 12 months following TBI, suggesting that determining APOE genotype will be of little prognostic value when predicting cognitive outcome following TBI. However, there was tentative evidence that possession of APOE ε4 was associated with

reduced executive function in our sample. While this might indicate that APOE ε4 is associated with a domain-specific effect, in the absence of supporting evidence from other studies to date, this finding must be interested cautiously and may be spurious.

It is acknowledged that although including mild TBI participants in the current research provided an ecologically valid investigation, it is possible that APOE ε4 exerts a detrimental effect in moderate to severe TBI, and furthermore, may have a region-specific effect that translates specifically to memory impairment. As such, focussing on moderate to severe TBI, and/or inclusion of measures of memory could prove fruitful for future researchers. This thesis also revealed the need for a more rigorous and focussed exploration of the relationship between APOE genotype and post-injury outcomes. In particular, gene x environment and gene x gene interactions, alongside the investigation of the impact (if any) of APOE ε2 and dose-dependency, may prove enlightening. In addition, the impact of missing data was explored and it was demonstrated that when missing data is ignored or compensated for using single imputation approaches, the true variability may be attenuated, whereas emerging approaches such as multiple imputation appear to better compensate for missingness. It is therefore important that researchers are aware not only of the potential impact of missing data, but also keep up-to-date with new generation analyses, and incorporate them into research as software becomes available. If this occurs, along with consistent use of core neuropsychological measures to assess cognition and reporting of data by genotype, then it is likely that research will be more robust and clarify the impact of APOE in TBI populations.

References

References marked with an asterisk indicate studies included in the meta-analyses.

Adibhatla, R. M., & Hatcher, J. F. (2007). Role of lipids in brain injury and diseases. *Future Lipidology, 2*(4), 403-422. doi: 10.2217/17460875.2.4.403

Alexander, S., Kerr, M. E., Kim, Y., Kamboh, M. I., Beers, S. R., & Conley, Y. P. (2007a). Apolipoprotein ε4 allele presence and functional outcome after severe traumatic brain injury. *Journal of Neurotrauma, 24*(5), 790-797. doi: 10.1089/neu/2006.0133

Alexander, D. M., Williams, L. M., Gatt, J. M., Dobson-Stone, C., Kuan, S. A., Todd, E. G., . . . Gordon, E. (2007b). The contribution of apolipoprotein E alleles on cognitive performance and dynamic neural activity over six decades. *Biological Psychology, 75*(3), 229-238. doi:10.1016/j.biopsycho.2007.03.001

Alfieri, A., Unterhuber, V., Pircher, M., Schwarz, A., Gazzeri, R., Reinert, M., & Widmer, H. R. (2008). Psychosocial and neurocognitive performance after spontaneous nonaneurysmal subarachnoid hemorrhage related to the APOE-epsilon 4 genotype: a prospective 5-year follow-up study. *Journal of Neurosurgery, 109*(6), 1019-1026. doi:10.3171/Jns.2008.109.12.1019

Allison, P. D. (2002). *Missing data*. Thousand Oaks, CA: Sage.

Altmann, A., Tian, L., Henderson, V. W., & Greicius, M. D. (2014). Sex modifies the APOE-related risk of developing Alzheimer disease. *Annals of Neurology, 75*(4), 563-573. doi:10.1002/ana.24135

American Psychological Association (2012). *Publication manual of the American Psychological Association* (6 ed.). Washington DC: Author.

* Anderson, G. D., Temkin, N. R., Dikmen, S. S., Diaz-Arrastia, R., Machamer, J. E., Farhrenbruch, C., . . . Sadrzadeh, S. M. H. (2009). Haptoglobin phenotype and

apolipoprotein E polymorphism: Relationship to posttraumatic seizures and neuropsychological functioning after traumatic brain injury. *Epilepsy & Behavior, 16*(3), 501-506. doi: 10.1016/j.yebeh.2009.08.025

* Ariza, M., Pueyo, R., Matarin, M. D., Junque, C., Mataro, M., Clemente, I., . . . Sahuquillo, J. (2006). Influence of APOE polymorphism on cognitive and behavioural outcome in moderate and severe traumatic brain injury. *Journal of Neurology Neurosurgery and Psychiatry, 77*(10), 1191-1193. doi: 10.1136/jnnp.2005.085167

Ashman, T. A., Cantor, J. B., Gordon, W. A., Sacks, A., Spielman, L., Egan, M., & Hibbard, M. R. (2008). A comparison of cognitive functioning in older adults with and without traumatic brain injury. *Journal of Head Trauma Rehabilitation, 23*(3), 139-148. doi: 10.1097/01.Htr.0000319930.69343.64

Azouvi, P. (2000). Neuroimaging correlates of cognitive and functional outcome after traumatic brain injury. *Current Opinion in Neurology, 13*(6), 665-669. doi: 10.1097/00019052-200012000-00009

Bagiella, E., Novack, T. A., Ansel, B., Diaz-Arrastia, R., Dikmen, S., Hart, T., & Temkin, N. (2010). Measuring outcome in traumatic brain injury treatment trials: Recommendations from the Traumatic Brain Injury Clinical Trials Network. *Journal of Head Trauma Rehabilitation, 25*(5), 375-382. doi: 10.1097/HTR.0b013e3181d27fe3

Bales, K. R., Liu, F., Wu, S., Lin, S. Z., Koger, D., DeLong, C., . . . Paul, S. M. (2009). Human APOE Isoform-Dependent Effects on Brain beta-Amyloid Levels in PDAPP Transgenic Mice. *Journal of Neuroscience, 29*(21), 6771-6779. doi:10.1523/Jneurosci.0887-09.2009

Balestreri, M., Czosnyka, M., Chatfield, D. A., Steiner, L. A., Schmidt, E. A., Smielewski, P., . . . Pickard, J. D. (2004). Predictive value of Glasgow coma scale after brain trauma:

change in trend over the past ten years. *Journal of Neurology Neurosurgery and Psychiatry, 75*(1), 161-162.

Baraldi, A. N., & Enders, C. K. (2010). An introduction to modern missing data analyses. *Journal of School Psychology, 48*(1), 5-37. doi: 10.1016/j.jsp.2009.10.001

Bartres-Faz, D., Junque, C., Moral, P., Lopez-Alomar, A., Sanchez-Aldeguer, J., & Clemente, I. C. (2002). Apolipoprotein E gender effects on cognitive performance in age-associated memory impairment. *Journal of Neuropsychiatry and Clinical Neurosciences, 14*(1), 80-83. doi:10.1176/appi.neuropsych.14.1.80

Bazarian, J. J., Cernak, I., Noble-Haeusslein, L., Potolicchio, S., & Temkin, N. (2009). Long-term Neurologic Outcomes After Traumatic Brain Injury. *Journal of Head Trauma Rehabilitation, 24*(6), 439-451.

Becker, J. T., Martinson, J. J., Penugonda, S., Kingsley, L., Molsberry, S., Reynolds, S., . . . Sacktor, N. (2015). No association between Apo epsilon 4 alleles, HIV infection, age, neuropsychological outcome, or death. *Journal of Neurovirology, 21*(1), 24-31. doi:10.1007/s13365-014-0290-2

Bendlin, B. B., Ries, M. L., Lazar, M., Alexander, A. L., Dempsey, R. J., Rowley, H. A., . . . Johnson, S. C. (2008). Longitudinal changes in patients with traumatic brain injury assessed with diffusion-tensor and volumetric imaging. *Neuroimage, 42*(2), 503-514. doi:10.1016/j.neuroimage.2008.04.254

Benton, A. L., & Hamsher, K. D. (1989). *Multilingual aphasia examination*. Iowa City: AJA Associates.

Beydoun, M. A., Boueiz, A., Abougergi, M. S., Kitner-Triolo, M. H., Beydoun, H. A., Resnick, S. M., . . . Zonderman, A. B. (2012). Sex differences in the association of the apolipoprotein E epsilon 4 allele with incidence of dementia, cognitive impairment,

and decline. *Neurobiology of Aging, 33*(4) 720-731. doi:72010.1016/j.neurobiolaging.2010.05.017

Bigler, E. D. (2013). Traumatic brain injury, neuroimaging, and neurodegeneration. *Frontiers in Human Neuroscience, 7*. doi:10.3389/Fnhum.2013.00395

Blackman, J. A., Worley, G., & Strittmatter, W. J. (2005). Apolipoprotein E and brain injury: implications for children. *Developmental Medicine and Child Neurology, 47*(1), 64-70. doi: 10.1017/S0012162205000113

Borkowski, J. G., Benton, A. L., & Spreen, O. (1967). Word fluency and brain damage. *Neuropsychologia, 5*, 135-140.

Borroni, B., Perani, D., Archetti, S., Agosti, C., Paghera, B., Bellelli, G., . . . Padovani, A. (2006). Functional correlates of Apolipoprotein E genotype in Frontotemporal Lobar Degeneration. *Bmc Neurology, 6*. Article 31 doi:10.1186/1471-2377-6-31

Botteri, M., Bandera, E., Minelli, C., & Latronico, N. (2008). Cerebral blood flow thresholds for cerebral ischemia in traumatic brain injury. A systematic review. *Critical Care Medicine, 36*(11), 3089-3092. doi: 10.1097/Ccm.0b013e31818bd7df

Bour, A., Rasquin, S. M. C., Baars, L., van Boxtel, M. P. J., Visser, P. J., Limburg, M., & Verhey, F. R. J. (2010). The effect of the APOE-epsilon 4 allele and ACE-I/D polymorphism on cognition during a two-year follow-up in first-ever stroke patients. *Dementia and Geriatric Cognitive Disorders, 29*(6), 534-542. doi: 10.1159/000314678

Bunce, D., Anstey, K. J., Burns, R., Christensen, H., & Easteal, S. (2011). Does possession of apolipoprotein E epsilon 4 benefit cognitive function in healthy young adults? *Neuropsychologia, 49*(7), 1693-1697. doi: 10.1016/j.neuropsychologia.2011.02.042

Bunce, D., Bielak, A. A., Anstey, K. J., Cherbuin, N., Batterham, P. J., & Easteal, S. (2014). APOE genotype and cognitive change in young, middle-aged, and older adults living

in the community. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 69*(4), 379-386. doi:10.1093/gerona/glt103

Butterworth, P., Anstey, K., Jorm, A. F., & Rodgers, B. (2004). A community survey demonstrated cohort differences in the lifetime prevalence of self-reported head injury. *Journal of Clinical Epidemiology, 57*(7), 742-748. doi:10.1016/j.jclinepi.2003.10.015

Carter, A. J. R., & Nguyen, A. Q. (2011). Antagonistic pleiotropy as a widespread mechanism for the maintenance of polymorphic disease alleles. *Bmc Medical Genetics, 12*. 160-173 doi:10.1186/1471-2350-12-160

* Chamelian, L., Reis, M., & Feinstein, A. (2004). Six-month recovery from mild to moderate traumatic brain injury: the role of APOE-epsilon 4 allele. *Brain, 127*, 2621-2628. doi: 10.1093/Brain/Awh296

Chang, L., Andres, M., Sadino, J., Jiang, C. S., Nakama, H., Miller, E., & Ernst, T. (2011). Impact of apolipoprotein E [epsilon]4 and HIV on cognition and brain atrophy: Antagonistic pleiotropy and premature brain aging. *Neuroimage, 58*(4), 1017-1027. doi:http://dx.doi.org/10.1016/j.neuroimage.2011.07.010

Chang, L., Jiang, C., Cunningham, E., Buchthal, S., Douet, V., Andres, M., & Ernst, T. (2014). Effects of APOE epsilon 4, age, and HIV on glial metabolites and cognitive deficits. *Neurology, 82*(24), 2213-2222. doi:10.1212/wnl.0000000000000526

Chen, X. H., Siman, R., Iwata, A., Meaney, D. F., Trojanowsko, J. Q., & Smith, D. H. (2004). Long-term accumulation of amyloid-beta, beta-secretase, presenilin-1, and caspase-3 in damaged axons following brain trauma. *American Journal of Pathology, 165*(2), 357-371. doi:10.1016/s0002-9440(10)63303-2

Corbo, R. M., & Scacchi, R. (1999). Apolipoprotein E (APOE) allele distribution in the world. Is APOE*4 a 'thrifty' allele? *Annals of Human Genetics, 63,* 301-310.

Corder, E. H., Saunders, A. M., Risch, N. J., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., . . . Pericakvance, M. A. (1994). Protective effect of Apolipoprotein-E type-2 allele for late-onset Alzheimer-disease. *Nature Genetics, 7*(2), 180-184. doi: 10.1038/Ng0694-180

Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., . . . Pericakvance, M. A. (1993). Gene dose of Apolipoprotein-E type-4 allele and the risk of Alzheimers-disease in late-onset families. *Science, 261*(5123), 921-923. doi: 10.1126/science.8346443

Corona, A., & Landreth, G. (2015). Lipidated APOE has effects on cognitive function that are independent of amyloid-beta pathology. *Brain, 138*, 3470-3472. doi:10.1093/brain/awv300

Coughlan, A. K., & Hollows, S. E. (1985). *The adult memory and information processing battery (AMIPB): Manual.* England: St James' University Hospital, Leeds.

* Crawford, F. C., Vanderploeg, R. D., Freeman, M. J., Singh, S., Waisman, M., Michaels, L., . . . Mullan, M. J. (2002). APOE genotype influences acquisition and recall following traumatic brain injury. *Neurology, 58*(7), 1115-1118.

Cudaback, E., Yang, Y., Montine, T. J., & Keene, C. D. (2015). APOE Genotype-Dependent Modulation of Astrocyte Chemokine CCL3 Production. *Glia, 63*(1), 51-65. doi:10.1002/glia.22732

Cumming, G. (2012). *Understanding the new statistics : Effect sizes, confidence intervals, and meta-analysis*. New York: Routledge.

Cumming, G. (2014). The new statistics: Why and how. *Psychological Science, 25*(1), 7-29. doi: 10.1177/0956797613504966

Dardiotis, E., Grigoriadis, S., & Hadjigeorgiou, G. M. (2012). Genetic factors influencing outcome from neurotrauma. *Current Opinion in Psychiatry, 25*(3), 231-238. doi: 10.1097/Yco.0b013e3283523c0e

Dean, P. J. A., & Sterr, A. (2013). Long-term effects of mild traumatic brain injury on cognitive performance. *Frontiers in Human Neuroscience, 7*. doi:10.3389/fnhum.2013.00030

Deary, I. J., Whalley, L. J., St Clair, D., Breen, G., Leaper, S., Lemmon, H., . . . Starr, J. M. (2003). The influence of the epsilon 4 allele of the apolipoprotein E gene on childhood IQ, nonverbal reasoning in old age, and lifetime cognitive change. *Intelligence, 31*(1), 85-92. doi:Pii S0160-2896(02)00114-9

Delacourte, A., David, J. P., Sergeant, N., Buee, L., Wattez, A., Vermersch, P., . . . Di Menza, C. (1999). The biochemical pathway of neurofibrillary degeneration in aging and Alzheimer's disease. *Neurology, 52*(6), 1158-1165.

de Oliveira Thais, M., Cavallazzi, G., Formolo, D. A., de Castro, L. D., Schmoeller, R., Guarnieri, R., . . . Walz, R. (2014). Limited predictive power of hospitalization variables for long-term cognitive prognosis in adult patients with severe traumatic brain injury. *Journal of Neuropsychology, 8*(1), 125-139. doi: 10.1111/jnp.12000

Dick, D. M., Agrawal, A., Keller, M. C., Adkins, A., Aliev, F., Monroe, S., . . . Sher, K. J. (2015). Candidate gene-environment interaction research: Reflections and recommendations. *Perspectives on Psychological Science, 10*(1), 37-59. doi: 10.1177/1745691614556682

Dikmen, S. S., Machamer, J. E., Winn, H. R., & Temkin, N. R. (1995). Neuropsychological outcome at 1-year post head-injury. *Neuropsychology, 9*(1), 80-90.

Donohoe, G. G., Salomaki, A., Lehtimaki, T., Pulkki, K., & Kairisto, V. (1999). Rapid identification of Apolipoprotein E genotypes by multiplex amplification refractory mutation system PCR and capillary gel electrophoresis. *Clinical Chemistry, 45*(1), 143-146.

Double, K. L., Halliday, G. M., Kril, J. J., Harasty, J. A., Cullen, K., Brooks, W. S., . . . Broe, G. A. (1996). Topography of brain atrophy during normal aging and Alzheimer's disease. *Neurobiology of Aging, 17*(4), 513-521.

Draper, K., & Ponsford, J. (2008). Cognitive functioning ten years following traumatic brain injury and rehabilitation. *Neuropsychology, 22*(5), 618-625. doi: 10.1037/0894-4105.22.5.618

Duncan, L. E., & Keller, M. C. (2011). A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. *American Journal of Psychiatry, 168*(10), 1041-1049. doi: 10.1176/appi.ajp.2011.11020191

Eisenberg, D. T. A., Kuzawa, C. W., & Hayes, M. G. (2010). Worldwide allele frequencies of the human Apolipoprotein E gene: Climate, local adaptations, and evolutionary history. *American Journal of Physical Anthropology, 143*(1), 100-111. doi: 10.1002/Ajpa.21298

Enders, C. K. (2010). *Applied missing data analysis* (1st ed.). New York: The Guildford Press.

Enders, C. K. (2011). Analyzing longitudinal data with missing values. *Rehabilitation Psychology, 56*(4), 267-288. doi: 10.1037/A0025579

Engelborghs, S., Dermaut, B., Marien, P., Symons, A., Vloeberghs, E., Maertens, K., . . . De Deyn, P. P. (2006). Dose dependent effect of APOE epsilon 4 on behavioral symptoms in frontal lobe dementia. *Neurobiology of Aging, 27*(2), 285-292. doi: 10.1016/j.neurobiolaging.2005.02.005

Eramudugolla, R., Bielak, A. A. M., Bunce, D., Easteal, S., Cherbuin, N., & Anstey, K. J. (2014). Long-term cognitive correlates of traumatic brain injury across adulthood and interactions with APOE genotype, sex, and age cohorts. *Journal of the International Neuropsychological Society, 20*(4), 444-454. doi: 10.1017/S1255617714000174

Fagerholm, E. D., Hellyer, P. J., Scott, G., Leech, R., & Sharp, D. J. (2015). Disconnection of network hubs and cognitive impairment after traumatic brain injury. *Brain, 138,* 1696-1709. doi:10.1093/brain/awv075

Farrer, L. A., Cupples, L. A., Haines, J. L., Hyman, B., Kukull, W. A., Mayeux, R., . . . vanDuijn, C. M. (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease - A meta-analysis. *Journal of the American Medical Association, 278*(16), 1349-1356.

Federoff, M., Jimenez-Rolando, B., Nalls, M. A., & Singleton, A. B. (2012). A large study reveals no association between APOE and Parkinson's disease. *Neurobiology of Disease, 46*(2), 389-392. doi:10.1016/j.nbd.2012.02.002

Ferguson, C. J. (2009). An effect size primer: A guide for clinicians and researchers. *Professional Psychology-Research and Practice, 40*(5), 532-538. doi:10.1037/a0015808

Field, A. (2013). *Discovering Statistics Using SPSS* (4th ed.). London: Sage.

Finnie, J. W. (2013). Neuroinflammation: Beneficial and detrimental effects after traumatic brain injury. *Inflammopharmacology, 21*(4), 309-320. doi:10.1007/s10787-012-0164-2

Fitz, N. F., Tapias, V., Cronican, A. A., Castranio, E. L., Saleem, M., Carter, A. Y., . . . Koldamova, R. (2015). Opposing effects of Apoe/Apoa1 double deletion on amyloid-beta pathology and cognitive performance in APP mice. *Brain, 138,* 3699-3715. doi:10.1093/brain/awv293

Fleisher, A., Grundman, M., Jack, C. R., Petersen, R. C., Taylor, C., Kim, H. T., . . . Alzheimer's Disease Cooperative Study. (2005). Sex, apolipoprotein E epsilon 4 status, and hippocampal volume in mild cognitive impairment. *Archives of Neurology, 62*(6), 953-957.

Fortune, N., & Wen, X. (1999). *The definition, incidence and prevalence of acquired brain injury in Australia.* Canberra: Australian Institute of Health and Welfare.

Friedman, G., Froom, P., Sazbon, L., Grinblatt, I., Shochina, M., Tsenter, J., . . . Groswasser, Z. (1999). Apolipoprotein E-epsilon 4 genotype predicts a poor outcome in survivors of traumatic brain injury. *Neurology, 52*(2), 244-248.

Frost, R. B., Farrer, T. J., Primosch, M., & Hedges, D. W. (2013). Prevalence of traumatic brain injury in the general adult population: A meta-analysis. *Neuroepidemiology, 40*(3), 154-159. doi: 10.1159/000343275

Ghebremedhin, E., Schultz, C., Thal, D. R., Rub, U., Ohm, T. G., Braak, E., & Braak, H. (2001). Gender and age modify the association between APOE and AD-related neuropathology. *Neurology, 56*(12), 1696-1701.

Gold, E. B. (2011). The timing of the age at which natural menopause occurs. *Obstetrics and Gynecology Clinics of North America, 38*(3), 425-440. doi:10.1016/j.ogc.2011.05.002

Graham, J. W. (2009). Missing data analysis: Making it work in the real world. *Annual Review of Psychology, 60,* 549-576. doi: 10.1146/annurev.psych.58.110405.085530

Graham, J. W. (2012). *Missing data: Analysis and design*. New York: Springer.

Graham, J. W., Olchowski, A. E., & Gilreath, T. D. (2007). How many imputations are really needed? - Some practical clarifications of multiple imputation theory. *Prevention Science, 8*(3), 206-213. doi: 10.1007/s11121-007-0070-9

Graham, J. W., & Schafer, J. L. (1999). On the performance of multiple imputation for multivariate data with small sample size. In R. H. Hoyle (Ed.), *Statistical strategies for small sample research* (pp. 1-29). California: Sage Publications.

Greenwood, P. M., Espeseth, T., Lin, M. K., Reinvang, I., & Parasuraman, R. (2014). Longitudinal change in working memory as a function of APOE genotype in midlife and old age. *Scandinavian Journal of Psychology, 55*(3), 268-277. doi:10.1111/sjop.12123

Greve, M. W., & Zink, B. J. (2009). Pathophysiology of traumatic brain injury. *Mount Sinai Journal of Medicine, 76*(2), 97-104. doi: 10.1002/Msj.20104

Gronwall, D., & Wrightson, P. (1974). Delayed recovery of intellectual function after minor head injury. *Lancet, 2*(7881), 605-609.

Han, S. D., & Bondi, M. W. (2008). Revision of the apolipoprotein E compensatory mechanism recruitment hypothesis. *Alzheimers & Dementia, 4*(4), 251-254. doi: 10.1016/j.jalz.2008.02.006

* Han, S. D., Drake, A. I., Cessante, L. M., Jak, A. J., Houston, W. S., Delis, D. C., . . . Bondi, M. W. (2007). Apolipoprotein E and traumatic brain injury in a military population: evidence of a neuropsychological compensatory mechanism? *Journal of Neurology Neurosurgery and Psychiatry, 78*(10), 1103-1108. doi: 10.1136/jnnp.2006.108183

Han, S. H., & Chung, S. Y. (2000). Marked hippocampal neuronal damage without motor deficits after mild concussive-like brain injury in apolipoprotein E-deficient mice. *Vascular Factors in Alzheimer's Disease, 903*, 357-365.

Harrington, K. D., Lim, Y. Y., Ellis, K. A., Copolov, C., Darby, D., Weinborn, M., . . . Maruff, P. (2013). The association of A beta amyloid and composite cognitive

measures in healthy older adults and MCI. *International Psychogeriatrics, 25*(10), 1667-1677. doi:10.1017/s1041610213001087

Harrison, J. E., Berry, J. G., & Jamieson, L. M. (2012). Head and traumatic brain injuries among Australian youth and young adults, July 2000-June 2006. *Brain Injury, 26*(7-8), 996-1004. doi:10.3109/02699052.2012.660515

Hartman, R. E., Laurer, H., Longhi, L., Bales, K. R., Paul, S. M., McIntosh, T. K., & Holtzman, D. M. (2002). Apolipoprotein E4 influences amyloid deposition but not cell loss after traumatic brain injury in a mouse model of Alzheimer's disease. *Journal of Neuroscience, 22*(23), 10083-10087

Heise, V., Filippini, N., Ebmeier, K. P., & Mackay, C. E. (2011). The APOE epsilon 4 allele modulates brain white matter integrity in healthy adults. *Molecular Psychiatry, 16*(9), 908-916. doi:10.1038/mp.2010.90

Heise, V., Filippini, N., Trachtenberg, A. J., Suri, S., Ebmeier, K. P., & Mackay, C. E. (2014). Apolipoprotein E genotype, gender and age modulate connectivity of the hippocampus in healthy adults. *Neuroimage, 98*, 23-30. doi:10.1016/j.neuroimage.2014.04.081

Henry, J. D., & Crawford, J. R. (2004). A meta-analytic review of verbal fluency performance in patients with traumatic brain injury. *Neuropsychology, 18*(4), 621-628. doi: http://dx.doi.org/10.1037/0894-4105.18.4.621

* Hodgkinson, A., Gillett, L., & Simpson, G. K. (2009). Does apolipoprotein E play a role in outcome after severe traumatic brain injury? *Brain Impairment, 10*(2), 162-168.

Honea, R. A., Vidoni, E., Harsha, A., & Burns, J. M. (2009). Impact of APOE on the healthy aging brain: A voxel-based MRI and DTI study. *Journal of Alzheimers Disease, 18*(3), 553-564. doi:10.3233/jad-2009-1163

Hoofien, D., Gilboa, A., Vakil, E., & Donovick, P. J. (2001). Traumatic brain injury (TBI) 10-20 years later: A comprehensive outcome study of psychiatric symptomatology, cognitive abilities and psychosocial functioning. *Brain Injury, 15*(3), 189-209.

Horsburgh, K., Graham, D. I., Stewart, J., & Nicoll, J. A. (1999). Influence of apolipoprotein E genotype on neuronal damage and apoE immunoreactivity in human hippocampus following global ischemia. *Journal of Neuropathology and Experimental Neurology, 58*(3), 227-234.

Horsburgh, K., Macrae, I. M., & Carswell, H. (2002). Estrogen is neuroprotective via an apolipoprotein E-dependent mechanism in a mouse model of global ischemia. *Journal of Cerebral Blood Flow and Metabolism, 22*(10), 1189-1195.

Horsburgh, K., McCarron, M. O., White, F., & Nicoll, J. A. R. (2000). The role of apolipoprotein E in Alzheimer's disease, acute brain injury and cerebrovascular disease: evidence of common mechanisms and utility of animal models. *Neurobiology of Aging, 21*(2), 245-255.

Horsburgh, K., McColl, B. W., White, F., & McCulloch, J. (2003). Apolipoprotein E influences neuronal death and repair. In K. Abe (Ed), *International Congress Series: Book 1252. Molecular mechanisms and epochal therapeutics of ischemic stroke and dementia: Invited papers from the International Symposium on Molecular Mechanism... 2002,* (pp. 171–178). Amsterdam, the Netherlands: Elsevier

Hostage, C. A., Choudhury, K. R., Doraiswamy, P. M., & Petrella, J. R. (2014). Mapping the effect of the Apolipoprotein E genotype on 4-year atrophy rates in an Alzheimer disease-related brain network. *Radiology, 271*(1), 211-219. doi:10.1148/radiol.13131041

Hostage, C. A., Choudhury, K. R., Doraiswamy, P. M., Petrella, J. R., & Alzheimer's Disease Neuroimaging. (2013). Dissecting the gene dose-effects of the APOE epsilon 4 and epsilon 2 alleles on hippocampal volumes in aging and Alzheimer's disease. *Plos One, 8*(2), 1-8. doi: 10.1371/journal.pone.0054483

Howell, D. C. (2013). *Statistical methods for psychology* (8 ed.). Canada: Wadsworth Cengage Learning.

Hoyt, L. T., & Falconi, A. M. (2015). Puberty and perimenopause: Reproductive transitions and their implications for women's health. *Social Science & Medicine, 132*, 103-112. doi:10.1016/j.socscimed.2015.03.031

Hyder, A. A., Wunderlich, C. A., Puvanachandra, P., Gururaj, G., & Kobusingye, O. C. (2007). The impact of traumatic brain injuries: A global perspective. *NeuroRehabilitation, 22*(5), 341-353.

Ignatius, M. J., Gebickeharter, P. J., Skene, J. H. P., Schilling, J. W., Weisgraber, K. H., Mahley, R. W., & Shooter, E. M. (1986). Expression of apolipoprotein-E during nerve degeneration and regeneration. *Proceedings of the National Academy of Sciences of the United States of America, 83*(4), 1125-1129.

Isoniemi, H., Tenovuo, O., Portin, R., Himanen, L., & Kairisto, V. (2006). Outcome of traumatic brain injury after three decades - Relationship to ApoE genotype. *Journal of Neurotrauma, 23*(11), 1600-1608. doi 10.1089/neu.2006.23.1600

Jack, C. R., Wiste, H. J., Weigand, S. D., Knopman, D. S., Vemuri, P., Mielke, M. M., . . . Petersen, R. C. (2015). Age, sex, and APOE epsilon 4 effects on memory, brain structure, and beta-amyloid across the adult life span. *Jama Neurology, 72*(5), 511-519. doi:10.1001/jamaneurol.2014.4821

Jacobs, B., Beems, T., van der Vliet, T. M., van Vugt, A. B., Hoedemaekers, C., Horn, J., . . . Vos, P. E. (2013). Outcome prediction in moderate and severe traumatic brain injury:

A focus on computed tomography variables. *Neurocritical Care, 19*(1), 79-89. doi: 10.1007/s12028-012-9795-9

Jaerve, A., & Muller, H. (2012). Chemokines in CNS injury and repair. *Cell and Tissue Research, 349*(1), 229-248. doi:10.1007/s00441-012-1427-3

Jansen, W. J., Ossenkoppele, R., Knol, D. L., Tijms, B. M., Scheltens, P., Verhey, F. R. J., . . . Grp, A. B. S. (2015). Prevalence of cerebral amyloid pathology in persons without dementia: A Meta-analysis. *Jama-Journal of the American Medical Association, 313*(19), 1924-1938. doi:10.1001/jama.2015.4668

Jasienska, G., Ellison, P. T., Galbarczyk, A., Jasienski, M., & Kalemba-Drozdz, M. (2015). Apolipoprotein E (ApoE) polymorphism is related to differences in potential fertility in women: a case of antagonistic pleiotropy? *Proceedings of the Royal Society. B, Biological sciences, 282*(1803), 20142395-20142395. doi:10.1098/rspb.2014.2395

Jelicic, H., Phelps, E., & Lerner, R. A. (2009). Use of missing data methods in longitudinal studies: The persistence of bad practices in developmental psychology. *Developmental Psychology, 45*(4), 1195-1199. doi: 10.1037/A0015665

Jelicic, H., Phelps, E., & Lerner, R. M. (2010). Why missing data matter in the longitudinal study of adolescent development: using the 4-H Study to understand the uses of different missing data methods. *Journal of Youth and Adolescence, 39*(7), 816-835. doi: 10.1007/s10964-010-9542-5

Jochemsen, H. M., Muller, M., van der Graaf, Y., & Geerlings, M. I. (2012). APOE epsilon 4 differentially influences change in memory performance depending on age. The SMART-MR study. *Neurobiology of Aging, 33*(4), 832.e15 - 832.e22. 1-12. doi: 10.1016/j.neurobiolaging.2011.07.016

Johnson, V. E., Stewart, J. E., Begbie, F. D., Trojanowski, J. Q., Smith, D. H., & Stewart, W. (2013). Inflammation and white matter degeneration persist for years after a single traumatic brain injury. *Brain, 136*, 28-42. doi:10.1093/brain/aws322

Jorm, A. F., Mather, K. A., Butterworth, P., Anstey, K. J., Christensen, H., & Easteal, S. (2007). APOE genotype and cognitive functioning in a large age-stratified population sample. *Neuropsychology, 21*(1), 1-8. doi: 2006-23022-001 [pii]10.1037/0894-4105.21.1.1

Kanekiyo, T., Xu, H. X., & Bu, G. J. (2014). ApoE and A beta in Alzheimer's disease: Accidental encounters or partners? *Neuron, 81*(4), 740-754. doi:10.1016/j.neuron.2014.01.045

Kang, J. E., Logroscino, G., De Vivo, I., Hunter, D., & Grodstein, F. (2005). Apolipoprotein E, cardiovascular disease and cognitive function in aging women. *Neurobiology of Aging, 26*(4), 475-484. doi:10.1016/j.neurobiolaging.2004.05.003

Kawai, N., Kawanishi, M., Kudomi, N., Maeda, Y., Yamamoto, Y., Nishiyama, Y., & Tamiya, T. (2013). Detection of brain amyloid beta deposition in patients with neuropsychological impairment after traumatic brain injury: PET evaluation using Pittsburgh Compound-B. *Brain Injury, 27*(9), 1026-1031. doi:10.3109/02699052.2013.794963

Karr, J. E., Areshenkoff, C. N., & Garcia-Barrera, M. A. (2013). The neuropsychological outcomes of concussion: A systematic review of meta-analyses on the cognitive sequelae of mild traumatic brain injury. *Neuropsychology, 28*(3), 321-336 doi: 10.1037/neu0000037

Kemp, S., Agostinis, A., House, A., & Coughlan, A. K. (2010). Analgesia and other causes of amnesia that mimic post-traumatic amnesia (PTA): A cohort study. *Journal of Neuropsychology, 4*, 231-236. doi:10.1348/174866409x482614

Kerr, M. E., Kamboh, M. L., Yookyung, K., Kraus, M. F., Puccio, A. M., DeKosky, S. T., & Marion, D. W. (2003). Relationship between apoE4 allele and excitatory amino acid levels after traumatic brain injury. *Critical Care Medicine, 31*(9), 2371-2379. doi: 10.1097/01.Ccm.0000080484.72004.C4

Kim, J., Basak, J. M., & Holtzman, D. M. (2009). The role of apolipoprotein E in Alzheimer's Disease. *Neuron, 63*(3), 287-303. doi:10.1016/j.neuron.2009.06.026

Kolakowsky-Hayner, S. A., Miner, K. D., & Kreutzer, J. S. (2001). Long-term life quality and family needs after traumatic brain injury. *Journal of Head Trauma Rehabilitation, 16*(4), 374-385. doi:10.1097/00001199-200108000-00007

Konrad, C., Geburek, A. J., Rist, F., Blumenroth, H., Fischer, B., Husstedt, I., . . . Lohmann, H. (2011). Long-term cognitive and emotional consequences of mild traumatic brain injury. *Psychological Medicine, 41*(6), 1197-1211. doi: 10.1017/S0033291710001728

Koutseff, A., Mittelhaeuser, C., Essabri, K., Auwerx, J., & Meziane, H. (2014). Impact of the apolipoprotein E polymorphism, age and sex on neurogenesis in mice: Pathophysiological relevance for Alzheimer's disease? *Brain Research, 1542*, 32-40. doi:10.1016/j.brainres.2013.10.003

Kulminski, A. M., Culminskaya, I., Arbeev, K. G., Ukraintseva, S. V., Stallard, E., Arbeeva, L., & Yashin, A. I. (2013). The role of lipid-related genes, aging-related processes, and environment in healthspan. *Aging Cell, 12*(2), 237-246. doi:10.1111/Acel.12046

Kulminski, A. M., Culminskaya, I., Ukraintseva, S. V., Arbeev, K. G., Arbeeva, L., Wu, D. Q., . . . Yashin, A. I. (2011). Trade-off in the effects of the apolipoprotein E polymorphism on the ages at onset of CVD and cancer influences human lifespan. *Aging Cell, 10*(3), 533-541. doi: 10.1111/j.1474-9726.2011.00689.x

Kumar, S., Rao, S. L., Chandramouli, B. A., & Pillai, S. (2013). Reduced contribution of executive functions in impaired working memory performance in mild traumatic brain injury patients. *Clinical Neurology and Neurosurgery, 115*(8), 1326-1332. doi:http://dx.doi.org/10.1016/j.clineuro.2012.12.038

Lambert, J. C., Coyle, N., & Lendon, C. (2004). The allelic modulation of apolipoprotein E expression by oestrogen: potential relevance for Alzheimer's disease. *Journal of Medical Genetics, 41*(2), 104-112. doi:10.1136/jmg.2003.005033

Lannsjo, M., Backheden, M., Johansson, U., af Geijerstam, J. L., & Borg, J. (2013). Does head CT scan pathology predict outcome after mild traumatic brain injury? *European Journal of Neurology, 20*(1), 124-129. doi: 10.1111/j.1468-1331.2012.03813.x

Laskowitz, D. T., Horsburgh, K., & Roses, A. D. (1998). Apolipoprotein E and the CNS response to injury. *Journal of Cerebral Blood Flow and Metabolism, 18*(5), 465-471.

Lawrence, D. W., Comper, P., Hutchison, M. G., & Sharma, B. (2015). The role of apolipoprotein e epsilon (ε)-4 allele on outcome following traumatic brain injury: A systematic review. *Brain Injury,* advance online publication. doi 10.3109/02699052.2015.1005131

Leibson, C. L., Brown, A. W., Long, K. H., Ransom, J. E., Mandrekar, J., Osler, T. M., & Malec, J. F. (2012). Medical care costs associated with traumatic brain injury over the full spectrum of disease: A controlled population-based study. *Journal of Neurotrauma, 29*(11), 2038-2049. doi:10.1089/neu.2010.1713

Leoni, V., Solomon, A., & Kivipelto, M. (2010). Links between ApoE, brain cholesterol metabolism, tau and amyloid beta-peptide in patients with cognitive impairment. *Biochemical Society Transactions, 38*, 1021-1025. doi: 10.1042/Bst0381021

Leroi, A. M., Bartke, A., De Benedictis, G., Franceschi, C., Gartner, A., Gonos, E., . . . Zwaan, B. (2005). What evidence is there for the existence of individual genes with

antagonistic pleiotropic effects? *Mechanisms of Ageing and Development, 126*(3), 421-429. doi: 10.1016/j.mad.2004.07.012

Levin, H. S., Odonnell, V. M., & Grossman, R. G. (1979). Galveston Orientation and Amnesia Test - practical scale to assess cognition after head-injury. *Journal of Nervous and Mental Disease, 167*(11), 675-684. doi:10.1097/00005053-197911000-00004

Levin, H. S., & Robertson, C. S. (2013). Mild traumatic brain injury in translation. *Journal of Neurotrauma, 30*(8), 610-617. doi:10.1089/neu.2012.2394

Levine, B., Fujiwara, E., O'Connor, C., Richard, N., Kovacevic, N., Mandic, M., . . . Black, S. E. (2006). In vivo characterization of traumatic brain injury neuropathology with structural and functional neuroimaging. *Journal of Neurotrauma, 23*(10), 1396-1411. doi:10.1089/neu.2006.23.1396

Lezak, M. D., Howieson, D. B., & Loring, D. W. (2004). *Neuropsychological assessment* (4th ed.). New York: Oxford University Press.

* Liberman, J. N., Stewart, W. F., Wesnes, K., & Troncoso, J. (2002). Apolipoprotein E epsilon 4 and short-term recovery from predominantly mild brain injury. *Neurology, 58*(7), 1038-1044.

* Lichtman, S. W., Seliger, G., Tycko, B., & Marder, K. (2000). Apolipoprotein E and functional recovery from brain injury following postacute rehabilitation. *Neurology, 55*(10), 1536-1539.

Lingsma, H. F., Roozenbeek, B., Steyerberg, E. W., Murray, G. D., & Maas, A. I. R. (2010). Early prognosis in traumatic brain injury: from prophecies to predictions. *The Lancet Neurology, 9*(5), 543-554.

Lipsey, M. W., & Wilson, D. B. (2001). *Practical meta-analysis*. Thousand Oaks, CA: Sage Publications.

Lipsky, R. H., Sparling, M. B., Ryan, L. M., Xu, K., Salazar, A. M., Goldman, D., & Warden, D. L. (2005). Association of COMT Val1.58Met genotype with executive functioning following traumatic brain injury. *Journal of Neuropsychiatry and Clinical Neurosciences, 17*(4), 465-471. doi:10.1176/appi.neuropsych.17.4.465

Louko, A. M., Vilkki, J., & Niskakangas, T. (2006). ApoE genotype and cognition after subarachnoid haemorrhage: a longitudinal study. *Acta Neurologica Scandinavica, 114*(5), 315-319. doi:10.1111/j.1600-0404.2006.00676.x

Lovell, M. R., Iverson, G. L., Collins, M. W., McKeag, D., & Maroon, J. C. (1999). Does loss of consciousness predict neuropsychological decrements after concussion? *Clinical Journal of Sport Medicine, 9*(4), 193-198. doi:10.1097/00042752-199910000-00002

Lynch, J. R., Morgan, D., Mance, J., Matthew, W. D., & Laskowitz, D. T. (2001). Apolipoprotein E modulates glial activation and the endogenous central nervous system inflammatory response. *Journal of Neuroimmunology, 114*(1-2), 107-113.

Lynch, J. R., Pineda, J. A., Morgan, D., Zhang, L., Warner, D. S., Benveniste, H., & Laskowitz, D. T. (2002). Apolipoprotein E affects the central nervous system response to injury and the development of cerebral edema. *Annals of Neurology, 51*(1), 113-117. doi: 10.1002/Ana.10098

Lynch, J. R., Tang, W., Wang, H. C., Vitek, M. P., Bennett, E. R., Sullivan, P. M., . . . Laskowitz, D. T. (2003). APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. *Journal of Biological Chemistry, 278*(49), 48529-48533. doi:10.1074/jbc.M306923200

Mace, B. E., Wang, H. C., Lynch, J. R., Moss, J., Sullivan, P., Colton, H., . . . Laskowitz, D. T. (2007). Apolipoprotein E modifies the CNS response to injury via a histamine-

mediated pathway. *Neurological Research, 29*(3), 243-250. doi:10.1179/016164107x158974

MacKenzie, J. D., Siddiqi, F., Babb, J. S., Bagley, L. J., Marmon, L. J., Sinson, G. P., & Grossman, R. I. (2002). Brain atrophy in mild or moderate traumatic brain injury: A longitudinal quantitative analysis. *American Journal of Neuroradiology, 23*(9), 1509-1515.

Mahley, R. W. (1988). Apolipoprotein-E - cholesterol transport protein with expanding role in cell biology. *Science, 240*(4852), 622-630.

Mahley, R. W., & Huang, Y. D. (1999). Apolipoprotein E: from atherosclerosis to Alzheimer's disease and beyond. *Current Opinion in Lipidology, 10*(3), 207-217. doi: 10.1097/00041433-199906000-00003

Mahley, R. W., & Huang, Y. (2006). Apolipoprotein (apo) E4 and Alzheimer's disease: unique conformational and biophysical properties of apoE4 can modulate neuropathology. *Acta Neurologica Scandinavica, 114*, 8-14.

Mahley, R. W., & Huang, Y. D. (2012). Apolipoprotein E sets the stage: Response to injury triggers neuropathology. *Neuron, 76*(5), 871-885. doi: 10.1016/j.neuron.2012.11.020

Mahley, R. W., & Rall, S. C. (2000). Apolipoprotein E: Far more than a lipid transport protein. *Annual Review of Genomics and Human Genetics, 1*, 507-537.

Malojcic, B., Mubrin, Z., Coric, B., Susnic, M., & Spilich, G. J. (2008). Consequences of mild traumatic brain injury on information processing assessed with attention and short-term memory tasks. *Journal of Neurotrauma, 25*(1), 30-37. doi: http://dx.doi.org/10.1089/neu.2007.0384

Manning, E. N., Barnes, J., Cash, D. M., Bartlett, J. W., Leung, K. K., Ourselin, S., & Fox, N. C. (2014). APOE epsilon 4 is associated with disproportionate progressive hippocampal atrophy in AD. *Plos One, 9*(5). doi: 10.1371/journal.pone.0097608

Mannix, R. C., Zhang, J., Park, J., Zhang, X., Bilal, K., Walker, K., . . . Whalen, M. J. (2011). Age-dependent effect of apolipoprotein E4 on functional outcome after controlled cortical impact in mice. *Journal of Cerebral Blood Flow and Metabolism, 31*(1), 351-361. doi: 10.1038/jcbfm.2010.99

Mannix, R., Meehan, W. P., Mandeville, J., Grant, P. E., Gray, T., Berglass, J., . . . Whalen, M. (2013). Clinical correlates in an experimental model of repetitive mild brain injury. *Annals of Neurology, 74*(1), 65-75. doi:10.1002/Ana.23858

Mar, J., Arrospide, A., Maria Begiristain, J., Larranaga, I., Elosegui, E., & Oliva-Moreno, J. (2011). The impact of acquired brain damage in terms of epidemiology, economics and loss in quality of life. *Bmc Neurology, 11*. Article 46 doi:10.1186/1471-2377-11-46

Marchant, N. L., King, S. L., Tabet, N., & Rusted, J. M. (2010). Positive effects of cholinergic stimulation favor young APOE epsilon 4 carriers. *Neuropsychopharmacology, 35*(5), 1090-1096. doi:10.1038/Npp.2009.214

Marklund, N., Farrokhnia, N., Hanell, A., Vanmechelen, E., Enblad, P., Zetterberg, H., . . . Hillered, L. (2014). Monitoring of beta-amyloid dynamics after human traumatic brain injury. *Journal of Neurotrauma, 31*(1), 42-55. doi:10.1089/neu.2013.2964

Masel, B. E., & DeWitt, D. S. (2010). Traumatic brain injury: A disease process, not an event. *Journal of Neurotrauma, 27*(8), 1529-1540. doi:10.1089/neu.2010.1358

Mathias, J. L., & Wheaton, P. (2007). Changes in attention and information-processing speed following severe traumatic brain injury: A meta-analytic review. *Neuropsychology, 21*(2), 212-223. doi: 10.1037/0894-4105.21.2.212

Mata, I. F., Leverenz, J. B., Weintraub, D., Trojanowski, J. Q., Hurtig, H. I., Van Deerlin, V. M., . . . Zabetian, C. P. (2014). APOE, MAPT, and SNCA genes and cognitive performance in Parkinson Disease. *Jama Neurology, 71*(11), 1405-1412. doi:10.1001/jamaneurol.2014.1455

Maxwell, S. S., Jackson, C. A., Paternoster, L., Cordonnier, C., Thijs, V., Al-Shahi Salman, R., & Sudlow, C. L. (2011). Genetic associations with brain microbleeds: Systematic review and meta-analyses. *Neurology, 77*(2), 158-167. doi: 10.1212/WNL.0b013e318224afa3

McAllister, T. W. (2010). Genetic factors modulating outcome after neurotrauma. *Physical Medicine and Rehabilitation, 2*(12), S241-S252. doi: 10.1016/j.pmrj.2010.10.005

McAllister, T. W., Tyler, A. L., Flashman, L. A., Rhodes, C. H., McDonald, B. C., Saykin, A. J., . . . Moore, J. H. (2012). Polymorphisms in the brain-derived neurotrophic factor gene influence memory and processing speed one month after brain injury. *Journal of Neurotrauma, 29*(6), 1111-1118. doi:10.1089/neu.2011.1930

McKnight, P. E., McKnight, K. M., Sidani, S., & Figueredo, A. J. (2007). *Missing data: A gentle introduction*. New York: The Guildford Press.

Menon, D. K., Schwab, K., Wright, D. W., Maas, A. I., & Common, I. I. I. (2010). Position statement: Definition of traumatic brain injury. *Archives of Physical Medicine and Rehabilitation, 91*(11), 1637-1640. doi:10.1016/j.apmr.2010.05.017

Methia, N., Andre, P., Hafezi-Moghadam, A., Economopoulos, M., Thomas, K. L., & Wagner, D. D. (2001). ApoE deficiency compromises the blood brain barrier especially after injury. *Molecular Medicine, 7*(12), 810-815.

Millar, K., Nicoll, J. A. R., Thornhill, S., Murray, G. D., & Teasdale, G. M. (2003). Long term neuropsychological outcome after head injury: relation to APOE genotype. *Journal of Neurology Neurosurgery and Psychiatry, 74*(8), 1047-1052.

Miller, M. A., Conley, Y., Scanlon, J. M., Ren, D. X., Kamboh, M. I., Niyonkuru, C., & Wagner, A. K. (2010). APOE genetic associations with seizure development after severe traumatic brain injury. *Brain Injury, 24*(12), 1468-1477. doi: 10.3109/02699052.2010.520299

Millis, S. R., Rosenthal, M., Novack, T. A., Sherer, M., Nick, T. G., Kreutzer, J. S., . . . Ricker, J. H. (2001). Long-term neuropsychological outcome after traumatic brain injury. *The Journal of Head Trauma Rehabilitation, 16*(4), 343-355.

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *British Medical Journal, 339*. doi: 10.1136/Bmj.B2535

Mondadori, C. R., de Quervain, D. J., Buchmann, A., Mustovic, H., Wollmer, M. A., Schmidt, C. F., . . . Henke, K. (2007). Better memory and neural efficiency in young apolipoprotein E epsilon4 carriers. *Cerebral Cortex, 17*(8), 1934-1947. doi:10.1093/cercor/bhl103

Moran, L. M., Taylor, H. G., Ganesalingam, K., Gastier-Foster, J. M., Frick, J., Bangert, B., . . . Yeates, K. O. (2009). Apolipoprotein E4 as a predictor of outcomes in pediatric mild traumatic brain injury. *Journal of Neurotrauma, 26*(9), 1489-1495. doi: 10.1089/neu.2008.0767

Morris, P. G., Wilson, J. T. L., Dunn, L. T., & Nicoll, J. A. R. (2004). Apolipoprotein E polymorphism and neuropsychological outcome following subarachnoid haemorrhage. *Acta Neurologica Scandinavica, 109*(3), 205-209. doi:10.1046/j.1600-0404.2003.00206.x

Morris, P. G., Wilson, J. T. L., Dunn, L. T., & Teasdale, G. M. (2005). Premorbid intelligence and brain injury. *British Journal of Clinical Psychology, 44*, 209-214. doi:10.1348/014466505x34174

Mortensen, E. L., & Hogh, P. (2001). A gender difference in the association between APOE genotype and age-related cognitive decline. *Neurology, 57*(1), 89-95.

Morton, M. V., & Wehman, P. (1995). Psychosocial and emotional sequelae of individuals with traumatic brain injury – a literature review and recommendations. *Brain Injury, 9*(1), 81-92. doi:10.3109/02699059509004574

Müller, K., Ingebrigtsen, T., Wilsgaard, T., Wikran, G., Fagerheim, T., Romner, B., & Waterloo, K. (2009). Prediction of time trends in recovery of cognitive function after mild head injury. *Neurosurgery, 64*(4), 698-704. doi: 10.1227/01.Neu.0000340978.42892.78

Mustafa, A. G., & Alshboul, O. A. (2013). Pathophysiology of traumatic brain injury. *Neurosciences (Riyadh), 18*(3), 222-234. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23887212

Myburgh, J. A., Cooper, D. J., Finfer, S. R., Venkatesh, B., Jones, D., Higgins, A., . . . Intensive, A. N. Z. (2008). Epidemiology and 12-month outcomes from traumatic brain injury in Australia and New Zealand. *Journal of Trauma-Injury Infection and Critical Care, 64*(4), 854-862. doi: 10.1097/Ta.0b013e3180340e77

Nathan, B. P., Barsukova, A. G., Shen, F., McAsey, M., & Struble, R. G. (2004). Estrogen facilitates neurite extension via apolipoprotein E in cultured adult mouse cortical neurons. *Endocrinology, 145*(7), 3065-3073. doi:10.1210/en.2003-1707

Nilsson, L. N. G., Arendash, G., Low, M., Costa, D., Roijani, A., Potter, H., . . . Paul, S. M. (2002). Cognitive impairment depends on ApoE and ACT-catalyzed amyloid formation in a mouse model of Alzheimer's disease. *Neurobiology of Aging, 23*(1), S199-S199.

Nilsson, L. G., Adolfsson, R., Backman, L., Cruts, M., Nyberg, L., Small, B. J., & Van Broeckhoven, C. (2006). The influence of APOE status on episodic and semantic

memory: Data from a population-based study. *Neuropsychology, 20*(6), 645-657. doi:10.1037/0894-4105.20.6.645

* Noé, E., Ferri, J., Colomer, C., Moliner, B., & Chirivella, J. (2010). APOE genotype and verbal memory recovery during and after emergence from post-traumatic amnesia. *Brain Injury, 24*(6), 886-892. doi: 10.3109/02699051003724952

Orihara, Y., & Nakasono, I. (2002). Induction of apolipoprotein E after traumatic brain injury in forensic autopsy cases. *International Journal of Legal Medicine, 116*(2), 92-98.

Ost, M., Nylen, K., Csajbok, L., Blennow, K., Rosengren, L., & Nellgard, B. (2008). Apolipoprotein E polymorphism and gender difference in outcome after severe traumatic brain injury. *Acta Anaesthesiologica Scandinavica, 52*(10), 1364-1369. doi:10.1111/j.1399-6576.2008.01675.x

Park, E., Bell, J. D., & Baker, A. J. (2008). Traumatic brain injury: Can the consequences be stopped? *Canadian Medical Association Journal, 178*(9), 1163-1170. doi:10.1503/Cmaj.080282

Perrin, P. B., Niemeier, J. P., Mougeot, J. L., Vannoy, C. H., Hirsch, M. A., Watts, J. A., . . . Whitney, M. P. (2015). Measures of injury severity and prediction of acute traumatic brain injury outcomes. *Journal of Head Trauma Rehabilitation, 30*(2), 136-142. doi: 10.1097/htr.0000000000000026

Poirier, J., Hess, M., May, P. C., & Finch, C. E. (1991). Astrocytic apolipoprotein-E mRNA and GFAP mRNA in hippocampus after entorhinal cortex lesioning. *Molecular Brain Research, 11*(2), 97-106.

Ponsford, J. (2013). Factors contributing to outcome following traumatic brain injury. *NeuroRehabilitation, 32*(4), 803-815. doi: 10.3233/nre-130904

Ponsford, J., McLaren, A., Schonberger, M., Burke, R., Rudzki, D., Olver, J., & Ponsford, M. (2011). The association between apolipoprotein E and traumatic brain injury severity and functional outcome in a rehabilitation sample. *Journal of Neurotrauma, 28*(9), 1683-1692. doi: 10.1089/neu.2010.1623

Ponsford, J., Olver, J., Ponsford, M., & Nelms, R. (2003). Long-term adjustment of families following traumatic brain injury where comprehensive rehabilitation has been provided. *Brain Injury, 17*(6), 453-468. doi:10.1080/0269905031000070143

Ponsford, J., Rudzki, D., Bailey, K., & Ng, K. T. (2007). Impact of apolipoprotein gene on cognitive impairment and recovery after traumatic brain injury. *Neurology, 68*(8), 619-620.

Ponsford, J., Willmott, C., Rothwell, A., Cameron, P., Kelly, A. M., Nelms, R., . . . Ng, K. (2000). Factors influencing outcome following mild traumatic brain injury in adults. *Journal of the International Neuropsychological Society, 6*(5), 568-579. doi:10.1017/s1355617700655066

Post, A., & Hoshizaki, T. B. (2012). Mechanisms of brain impact injuries and their prediction: A review. *Trauma, 14*(4), 327-349 doi:10.1177/1460408612446573

Proust-Lima, C., Dartigues, J. F., & Jacqmin-Gadda, H. (2011). Misuse of the linear mixed model when evaluating risk factors of cognitive decline. *American Journal of Epidemiology, 174*(9), 1077-1088. doi: 10.1093/Aje/Kwr243

Pruthi, N., Chandramouli, B. A., Kuttappa, T. B., Rao, S. L., Subbakrishna, D. K., Abraham, M. P., . . . Shankar, S. K. (2010). Apolipoprotein E polymorphism and outcome after mild to moderate traumatic brain injury: A study of patient population in India. *Neurology India, 58*(2), 264-269. doi: 10.4103/0028-3886.63810

Raber, J., Bongers, G., LeFevour, A., Buttini, M., & Mucke, L. (2002). Androgens protect against apolipoprotein E4-induced cognitive deficits. *Journal of Neuroscience, 22*(12), 5204-5209.

Raber, J., Wong, D., Buttini, M., Orth, M., Bellosta, S., Pitas, R. E., . . . Mucke, L. (1998). Isoform-specific effects of human apolipoprotein E on brain function revealed in ApoE knockout mice: Increased susceptibility of females. *Proceedings of the National Academy of Sciences of the United States of America, 95*(18), 10914-10919.

Rabinowitz, A. R., & Levin, H. S. (2014). Cognitive sequelae of traumatic brain injury. *Psychiatric Clinics of North America, 37*(1), 1-11. doi: 10.1016/j.psc.2013.11.004

Rapoport, M., Wolf, U., Herrmann, N., Kiss, A., Shammi, P., Reis, M., . . . Feinstein, A. (2008). Traumatic brain injury, apolipoprotein E-epsilon 4, and cognition in older adults: A two-year longitudinal study. *Journal of Neuropsychiatry and Clinical Neurosciences, 20*(1), 68-73.

Richter-Schmidinger, T., Alexopoulos, P., Horn, M., Maus, S., Reichel, M., Rhein, C., . . . Kornhuber, J. (2011). Influence of brain-derived neurotrophic-factor and apolipoprotein E genetic variants on hippocampal volume and memory performance in healthy young adults. *Journal of Neural Transmission, 118*(2), 249-257. doi:10.1007/s00702-010-0539-8

Rohling, M. L., Binder, L. M., Demakis, G. J., Larrabee, G. J., Ploetz, D. M., & Langhinrichsen-Rohling, J. (2011). A meta-analysis of neuropsychological outcome after mild traumatic brain injury: Re-analyses and reconsiderations of Binder et al. (1997), Frencham et al. (2005), and Pertab et al. (2009). *Clinical Neuropsychologist, 25*(4), 608-623. doi: 10.1080/13854046.2011.565076

Roozenbeek, B., Maas, A. I. R., & Menon, D. K. (2013). Changing patterns in the epidemiology of traumatic brain injury. *Nature Reviews Neurology, 9*(4), 231-236. doi:10.1038/nrneurol.2013.22

Roth, P. L. (1994). Missing data - a conceptual review for applied psychologists. *Personnel Psychology, 47*(3), 537-560. doi: 10.1111/j.1744-6570.1994.tb01736.x

Rubin, D. B. (1976). Inference and missing data. *Biometrika, 63*(3), 581-590. doi: 10.1093/biomet/63.3.581

Rusted, J. M., Evans, S. L., King, S. L., Dowell, N., Tabet, N., & Lofts, P. S. (2013). APOE e4 polymorphism in young adults is associated with improved attention and indexed by distinct neural signatures. *Neuroimage, 65*, 364-373. doi: 10.1016/j.neuroimage.2012.10.010

Sabo, T., Lomnitski, L., Nyska, A., Beni, S., Maronpot, R. R., Shohami, E., . . . Michaelson, D. M. (2000). Susceptibility of transgenic mice expressing human apolipoprotein E to closed head injury: The allele E3 is neuroprotective whereas E4 increases fatalities. *Neuroscience, 101*(4), 879-884.

Saunders, A. M., Strittmatter, W. J., Schmechel, D., St. George-Hyslop, P. H. S., Pericakvance, M. A., Joo, S. H., . . . Roses, A. D. (1993). Association of Apolipoprotein-E allele epsilon-4 with late-onset familial and sporadic Alzheimers-disease. *Neurology, 43*(8), 1467-1472.

Schafer, J. L., & Graham, J. W. (2002). Missing data: Our view of the state of the art. *Psychological Methods, 7*(2), 147-177. doi: 10.1037//1082-989x.7.2.147

Schafer, J. L., & Olsen, M. K. (1998). Multiple imputation for multivariate missing-data problems: A data analyst's perspective. *Multivariate Behavioral Research, 33*(4), 545-571. doi: 10.1207/s15327906mbr3304_5

Schretlen, D. J., Buffington, A. L. H., Meyer, S. M., & Pearlson, G. D. (2005). The use of word-reading to estimate "premorbid" ability in cognitive domains other than intelligence. *Journal of the International Neuropsychological Society, 11*(6), 784-787. doi 10.1017/S1355617705050939

Schmidt, O. I., Infanger, M., Heyde, C. E., Ertel, W., & Stahel, P. F. (2004). The role of neuroinflammation in traumatic brain injury. *European Journal of Trauma, 30*(3), 135-149. doi 10.1007/s00068-004-1394-9

Schonberger, M., Ponsford, J., Reutens, D., Beare, R., & O'Sullivan, R. (2009). The relationship between age, injury severity, and MRI findings after traumatic brain injury. *Journal of Neurotrauma, 26*(12), 2157-2167. doi: 10.1089/neu.2009.0939

Scott, J., Knott, T. J., Shaw, D. J., & Brook, J. D. (1985). Localization of genes encoding apolipoproteins Ci, Cii, and E to the P13-]Cen region of human chromosome-19. *Human Genetics, 71*(2), 144-146.

Senathi-Raja, D., Ponsford, J., & Schonberger, M. (2010). Impact of Age on Long-Term Cognitive Function After Traumatic Brain Injury. *Neuropsychology, 24*(3), 336-344. doi:10.1037/A0018239

* Shadli, R. M., Pieter, M. S., Yaacob, M. J., & Rashid, F. A. (2011). APOE genotype and neuropsychological outcome in mild-to-moderate traumatic brain injury: A pilot study. *Brain Injury, 25*(6), 596-603. doi: 10.3109/02699052.2011.572947

Shames, J., Treger, I., Ring, H., & Giaquinto, S. (2007). Return to work following traumatic brain injury: Trends and challenges. *Disability and Rehabilitation, 29*(17), 1387-1395. doi:10.1080/09638280701315011

Sherer, M., Sander, A. M., Maestas, K. L., Pastorek, N. J., Nick, T. G., & Li, J. Y. (2015). Accuracy of self-reported length of coma and posttraumatic amnesia in persons with

medically verified traumatic brain injury. *Archives of Physical Medicine and Rehabilitation, 96*(4), 652-658. doi: 10.1016/j.apmr.2014.10.024

Sherer, M., Struchen, M. A., Yablon, S. A., Wang, Y., & Nick, T. G. (2008). Comparison of indices of traumatic brain injury severity: Glasgow Coma Scale, length of coma and post-traumatic amnesia. *Journal of Neurology Neurosurgery and Psychiatry, 79*(6), 678-685. doi: 10.1136/jnnp.2006.111187

Shin, M. H., Kweon, S. S., Choi, J. S., Lee, Y. H., Nam, H. S., Park, K. S., . . . Jeong, S. K. (2014). The effect of an APOE polymorphism on cognitive function depends on age. *Journal of Neurology, 261*(1), 66-72. doi:10.1007/s00415-013-7157-y

Shinohara, M., Petersen, R. C., Dickson, D. W., & Bu, G. J. (2013). Brain regional correlation of amyloid-beta with synapses and apolipoprotein E in non-demented individuals: potential mechanisms underlying regional vulnerability to amyloid-beta accumulation. *Acta Neuropathologica, 125*(4), 535-547. doi:10.1007/s00401-013-1086-9

Shores, E. A., Marosszeky, J. E., Sandanam, J., & Batchelor, J. (1986). Preliminary validation of a clinical-scale for measuring the duration of posttraumatic amnesia. *Medical Journal of Australia, 144*(11), 569-572.

Sinharay, S., Stern, H. S., & Russell, D. (2001). The use of multiple imputation for the analysis of missing data. *Psychological Methods, 6*(4), 317-329. doi: 10.1037//1082-989x.6.4.317

Small, B. J., Rosnick, C. B., Fratiglioni, L., & Backman, L. (2004). Apolipoprotein E and cognitive performance: A meta-analysis. *Psychology and Aging, 19*(4), 592-600. doi: 10.1037/0882-7974.19.4.592

Smith, C., Gentleman, S. M., Leclercq, P. D., Murray, L. S., Griffin, W. S. T., Graham, D. I., & Nicoll, J. A. R. (2013). The neuroinflammatory response in humans after traumatic

brain injury. *Neuropathology and Applied Neurobiology*, *39*(6), 654-666. doi:10.1111/nan.12008

Smith, C., Graham, D. I., Murray, L. S., Stewart, J., & Nicoll, J. A. R. (2006). Association of APOE e4 and cerebrovascular pathology in traumatic brain injury. *Journal of Neurology Neurosurgery and Psychiatry*, *77*(3), 363-366. doi:10.1136/jnnp.2005.074617

Smith, D. H., Meaney, D. F., & Shull, W. H. (2003). Diffuse axonal injury in head trauma. *Journal of Head Trauma Rehabilitation, 18*(4), 307-316. doi:10.1097/00001199-200307000-00003

Snipes, G. J., Mcguire, C. B., Norden, J. J., & Freeman, J. A. (1986). Nerve injury stimulates the secretion of apolipoprotein-E by nonneuronal cells. *Proceedings of the National Academy of Sciences of the United States of America*, *83*(4), 1130-1134.

Spitz, G., Ponsford, J. L., Rudzki, D., & Maller, J. J. (2012). Association between cognitive performance and functional outcome following traumatic brain injury: A longitudinal multilevel examination. *Neuropsychology*, *26*(5), 604-612. doi: 10.1037/a0029239

Stoica, B. A., & Faden, A. I. (2010). Cell death mechanisms and modulation in traumatic brain injury. *Neurotherapeutics, 7*(1), 3-12.

Strauss, E., Sherman, E. M. S., & Spreen, O. (2006). *A compendium of neuropsychological tests: Administration, norms, and commentary* (3rd ed.). New York: Oxford University Press.

Strittmatter, W. J., Saunders, A. M., Schmechel, D., Pericakvance, M., Enghild, J., Salvesen, G. S., & Roses, A. D. (1993). Apolipoprotein-E - high-avidity binding to beta-amyloid and increased frequency of type-4 allele in late-onset familial Alzheimer-

disease. *Proceedings of the National Academy of Sciences of the United States of America, 90*(5), 1977-1981. doi: 10.1073/pnas.90.5.1977

Struble, R. G., Nathan, B. P., Cady, C., Cheng, X. Y., & McAsey, M. (2007). Estradiol regulation of astroglia and apolipoprotein E: An important role in neuronal regeneration. *Experimental Gerontology, 42*(1-2), 54-63. doi: 10.1016/j.exger.2006.05.013

Sundström, A., Marklund, P., Nilsson, L. G., Cruts, M., Adolfsson, R., Van Broeckhoven, C., & Nyberg, L. (2004). APOE influences on neuropsychological function after mild head injury - Within-person comparisons. *Neurology, 62*(11), 1963-1966.

Suri, S., Heise, V., Trachtenberg, A. J., & Mackay, C. E. (2013). The forgotten APOE allele: A review of the evidence and suggested mechanisms for the protective effect of APOE epsilon 2. *Neuroscience and Biobehavioral Reviews, 37*(10), 2878-2886. doi: 10.1016/j.neubiorev.2013.10.010

Tai, L. M., Ghura, S., Koster, K. P., Liakaite, V., Maienschein-Cline, M., Kanabar, P., . . . LaDu, M. J. (2015). APOE-modulated Abeta-induced neuroinflammation in Alzheimer's disease: current landscape, novel data, and future perspective. *Journal of Neurochemistry, 133*(4), 465-488. doi: 10.1111/jnc.13072

Tagliaferri, F., Compagnone, C., Korsic, M., Servadei, F., & Kraus, J. (2006). A systematic review of brain injury epidemiology in Europe. *Acta Neurochirurgica, 148*(3), 255-268. doi:10.1007/s00701-005-0651-y

Tate, R. L., Godbee, K., & Sigmundsdottir, L. (2013). A systematic review of assessment tools for adults used in traumatic brain injury research and their relationship to the ICF. *NeuroRehabilitation, 32*(4), 729-750. doi: 10.3233/NRE-130898

Teasdale, G. M., Murray, G. D., & Nicoll, J. A. R. (2005). The association between APOE epsilon 4, age and outcome after head injury: a prospective cohort study. *Brain, 128,* 2556-2561. doi:10.1093/Brain/Awh595

Teasdale, T. W., Jorgensen, O.S., Ripa, C., Nielsen, A. S., & Christensen, A. L. (2000). Apolipoprotein E and subjective symptomatology following brain injury rehabilitation. *Neuropsychological Rehabilitation, 10*(2), 151-166.

Thal, D. R., Rub, U., Orantes, M., & Braak, H. (2002). Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology, 58*(12), 1791-1800.

Tuminello, E. R., & Han, S. D. (2011). The apolipoprotein e antagonistic pleiotropy hypothesis: review and recommendations. *International Journal of Alzheimers Disease, 2011,* 1-12 doi: 10.4061/2011/726197

Tuominen, R., Joelsson, P., & Tenovuo, O. (2012). Treatment costs and productivity losses caused by traumatic brain injuries. *Brain Injury, 26*(13-14), 1697-1701. doi:10.3109/02699052.2012.722256

Ungar, L., Altmann, A., & Greicius, M. D. (2014). Apolipoprotein E, gender, and Alzheimer's disease: an overlooked, but potent and promising interaction. *Brain Imaging and Behavior, 8*(2), 262-273. doi:10.1007/s11682-013-9272-x

Utermann, G., Langenbeck, U., Beisiegel, U., & Weber, W. (1980). Genetics of the apolipoprotein-E system in man. *American Journal of Human Genetics, 32*(3), 339-347.

Vanderploeg, R. D., Curtiss, G., & Belanger, H. G. (2005). Long-term neuropsychological outcomes following mild traumatic brain injury. *Journal of the International Neuropsychological Society, 11*(3), 228-236. doi:http://dx.doi.org/10.1017/S1355617705050289

Verghese, P. B., Castellano, J. M., & Holtzman, D. M. (2011). Apolipoprotein E in Alzheimer's disease and other neurological disorders. *Lancet Neurology, 10*(3), 241-252.

Verhaeghe, S., Defloor, T., & Grypdonck, M. (2005). Stress and coping among families of patients with traumatic brain injury: a review of the literature. *Journal of Clinical Nursing, 14*(8), 1004-1012. doi:10.1111/j.1365-2702.2005.01126.x

Wagle, J., Farner, L., Flekkoy, K., Wyller, T. B., Sandvik, L., Eiklid, K. L., . . . Engedal, K. (2010). Cognitive impairment and the role of the ApoE epsilon 4-allele after stroke-a 13 months follow-up study. *International Journal of Geriatric Psychiatry, 25*(8), 833-842. doi:10.1002/Gps.2425

Wagner, A. K., Hatz, L. E., Scanlon, J. M., Niyonkuru, C., Miller, M. A., Ricker, J. H., . . . Ferrell, R. E. (2012). Association of KIBRA rs17070145 polymorphism and episodic memory in individuals with severe TBI. *Brain Injury, 26*(13-14), 1658-1669. doi: 10.3109/02699052.2012.700089

Walker, K. R., & Tesco, G. (2013). Molecular mechanisms of cognitive dysfunction following traumatic brain injury. *Frontiers in Aging Neuroscience, 5*. Article 29 doi:10.3389/Fnagi.2013.00029

Ward, D. D., Summers, M. J., Saunders, N. L., Janssen, P., Stuart, K. E., & Vickers, J. C. (2014). APOE and BDNF Val66Met polymorphisms combine to influence episodic memory function in older adults. *Behavioural Brain Research, 271*, 309-315. doi:10.1016/j.bbr.2014.06.022

Weaver, S. M., Chau, A., Portelli, J. N., & Grafman, J. (2012). Genetic polymorphisms influence recovery from traumatic brain injury. *Neuroscientist, 18*(6), 631-644. doi: 10.1177/1073858411435706

Weaver, S. M., Portelli, J. N., Chau, A., Cristofori, I., Moretti, L., & Grafman, J. (2014). Genetic polymorphisms and traumatic brain injury: the contribution of individual differences to recovery. *Brain Imaging and Behavior, 8*(3), 420-434. doi: 10.1007/s11682-012-9197-9

Wechsler, D. (1996). *Wechsler Adult Intelligences Scale III*. New York: Psychological Corporation.

Werner, C., & Engelhard, K. (2007). Pathophysiology of traumatic brain injury. *British Journal of Anaesthesia, 99*(1), 4-9. doi:10.1093/bja/aem131

Westlye, E. T., Hodneland, E., Haasz, J., Espeseth, T., Lundervold, A., & Lundervold, A. J. (2012). Episodic memory of APOE epsilon 4 carriers is correlated with fractional anisotropy, but not cortical thickness, in the medial temporal lobe. *Neuroimage, 63*(1), 507-516. doi:10.1016/j.neuroimage.2012.06.072

White, F., Nicoll, J. A. R., & Horsburgh, K. (2001). Alterations in apoE and apoJ in relation to degeneration and regeneration in a mouse model of entorhinal cortex lesion. *Experimental Neurology, 169*(2), 307-318. doi: 10.1006/exnr.2001.7655

White, F., Nicoll, J. A. R., Roses, A. D., & Horsburgh, K. (2001). Impaired neuronal plasticity in transgenic mice expressing human apolipoprotein E4 compared to E3 in a model of entorhinal cortex lesion. *Neurobiology of Disease, 8*(4), 611-625. doi:10.1006/nbdi.2001.0401

Wierenga, C. E., Clark, L. R., Dev, S. I., Shin, D. D., Jurick, S. M., Rissman, R. A., . . . Bondi, M. W. (2013). Interaction of age and APOE genotype on cerebral blood flow at rest. *Journal of Alzheimers Disease, 34*(4), 921-935. doi: 10.3233/Jad-121897

Wilde, E. A., Whiteneck, G. G., Bogner, J., Bushnik, T., Cifu, D. X., Dikmen, S., . . . von Steinbuechel, N. (2010). Recommendations for the use of common outcome measures

in traumatic brain injury research. *Archives of Physical Medicine and Rehabilitation, 91*(11), 1650-1660. doi: 10.1016/j.apmr.2010.06.033

Wilkinson, L., & American Psychological Task Force on Statistical Inference. (1999). Statistical methods in psychology journals: Guidelines and explanations. *American Psychologist, 54*(8), 594-604. doi: 10.1037/0003-066X.54.8.594

Wilson, R. S., Schneider, J. A., Barnes, L. L., Beckett, L. A., Aggarwal, N. T., Cochran, E. J., . . . Bennett, D. A. (2002). The apolipoprotein E epsilon 4 allele and decline in different cognitive systems during a 6-year period. *Archives of Neurology, 59*(7), 1154-1160. doi:10.1001/archneur.59.7.1154

Wisdom, N. M., Callahan, J. L., & Hawkins, K. A. (2011). The effects of apolipoprotein E on non-impaired cognitive functioning: A meta-analysis. *Neurobiology of Aging, 32*(1), 63-74. doi: 10.1016/j.neurobiolaging.2009.02.003

Wolf, A. B., Valla, J., Bu, G. J., Kim, J., LaDu, M. J., Reiman, E. M., & Caselli, R. J. (2013). Apolipoprotein E as a beta-amyloid-independent factor in Alzheimer's disease. *Alzheimers Research & Therapy, 5*(5), 8. doi:10.1186/1758-9193-5-38

Yang, S. T., Hsiao, I. T., Hsieh, C. J., Chiang, Y. H., Yen, T. C., Chiu, W. T., . . . Hu, C. J. (2015). Accumulation of amyloid in cognitive impairment after mild traumatic brain injury. *Journal of the Neurological Sciences, 349*(1-2), 99-104. doi:10.1016/j.jns.2014.12.032

Yin, J. X., Turner, G. H., Coons, S. W., Maalouf, M., Reiman, E. M., & Shi, J. (2014). Association of amyloid burden, brain atrophy and memory deficits in aged Apolipoprotein epsilon 4 mice. *Current Alzheimer Research, 11*(3), 283-290.

Yu, Y. W., Lin, C. H., Chen, S. P., Hong, C. J., & Tsai, S. J. (2000). Intelligence and event-related potentials for young female human volunteer apolipoprotein E epsilon4 and non-epsilon4 carriers. *Neuroscience Letters, 294*(3), 179-181.

Yue, J. K., Pronger, A. M., Ferguson, A. R., Temkin, N. R., Sharma, S., Rosand, J., . . . Investigators, T.-T. (2015). Association of a common genetic variant within ANKK1 with six-month cognitive performance after traumatic brain injury. *Neurogenetics, 16*(3), 169-180. doi:10.1007/s10048-015-0437-1

Yuh, E. L., Mukherjee, P., Lingsma, H. F., Yue, J. K., Ferguson, A. R., Gordon, W. A., . . . TRACK-TBI Investigators. (2013). Magnetic resonance imaging improves 3-month outcome prediction in mild traumatic brain injury. *Annals of Neurology, 73*(2), 224-235. doi:10.1002/ana.23783

Zannis, V. I., Just, P. W., & Breslow, J. L. (1981). Human apolipoprotein-E isoprotein subclasses are genetically-determined. *American Journal of Human Genetics, 33*(1), 11-24.

Zeng, S., Jiang, J. X., Xu, M. H., Xu, L. S., Shen, G. J., Zhang, A. Q., & Wang, X. H. (2014). Prognostic value of Apolipoprotein E epsilon4 allele in patients with traumatic brain injury: A meta-analysis and meta-regression. *Genetic Testing and Molecular Biomarkers, 18*(3), 202-210. doi: 10.1089/gtmb.2013.0421

Zhang, J. Y., Yoganandan, N., Pintar, F. A., & Gennarelli, T. A. (2006). Role of translational and rotational accelerations on brain strain in lateral head impact. *Biomedical Sciences Instrumentation, 42*, 501-506.

Zhou, W. D., Xu, D., Peng, X. X., Zhang, Q. H., Jia, J. P., & Crutcher, K. A. (2008). Meta-analysis of APOE4 allele and outcome after traumatic brain injury. *Journal of Neurotrauma, 25*(4), 279-290. doi: 10.1089/neu.2007.0489

Zuercher, M., Ummenhofer, W., Baltussen, A., & Walder, B. (2009). The use of Glasgow Coma Scale in injury assessment: A critical review. *Brain Injury, 23*(5), 371-384. doi: 10.1080/02699050902926267

Appendix A – Ethics Approvals, Information Sheets and Consent Forms

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Appendix B – Information for DNA analysis protocols

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Appendix C – Data analysis output for Chapters 2 - 6

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Appendix D – Padgett, Skilbeck, & Summers (2014) reprint

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